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THE MATLOCK BATH FORAY

31 *May*–3 *June* 1935

By J. RAMSBOTTOM

A party of fifteen assembled on Friday, 31 May, at the Matlock Bath Hotel. It was obvious that six weeks without rain had had a very adverse effect on the limestone soil from the point of view of a mycologist.

Cars were taken on the Saturday morning to the Ashbourne end of Dovedale, much of which is now the property of the National Trust. The day was ideal for a stroll by the Dove beloved by Isaac Walton and Charles Cotton, and the eyes of the fishermen were frequently on the stream "full of very good trout and grayling", as Piscator tells Viator. A fair number of rusts were found, some in good quantity, and *Synchytrium Mercurialis* occurred abundantly along the side of the path bordering the Dove.

The Sunday foray was to the woods on both sides of the curiously named Via Gellia,* the upper part of the valley being worked. Hardly had collecting started than the much-needed rain came down in torrents. Agarics were practically absent, and it soon became impossible to continue searching for microfungi in comfort or with profit. On Monday the sky was overcast, but the rain kept off until late in the afternoon. The middle part of Via Gellia (Middleton Wood) was visited, and an intensive search was made for microfungi. All the members present contributed to the subjoined list, particularly Dr Malcolm Wilson and Dr Alex Smith with rusts, and Mr T. Petch and Mr C. G. C. Chesters with Pyrenomycetes; Miss F. L. Stephens worked out some of the microfungi.

* "The road was built by Philip Gell, of Hopton Hall, near Wirksworth, about 1800, to open up the mineral resources of that part of his estates. It was named after the centurion, Phillipus Gellius, who in the early part of the fourth century was in charge of the band of Roman lead-miners on the surrounding hills. His inscribed urn, with the date, was found at Ivett's Low, just above the Via Gellia. Adjacent hills are named Soldier's Knoll and Chariot Clump, and are connected by Chariot Way.

"The name of Phillipus Gellius is also found on the Roman Wall (where he was at one time in charge of a cohort)." (*The Observer*, 19 Jan. 1936.)

"The name Viyella, given to a much advertised mixture of woollen and cotton which is a product of Derbyshire mills, is—*horresco referens*—a corruption of Via Gellia. Its only excuse is that it was perpetrated to satisfy the requirements of the Patent Office." (J. B. Firth.)

List of Species gathered during the Foray

D. = Dovedale; *G.* = Via Gellia; *MB.* = Matlock Bath.

HYMENOMYCETES

Armillaria mellea (Vahl) Fr., rhizomorphs only, *G.*
Tricholoma imbricatum Fr., *G.*
Omphalia umbellifera (Linn.) Fr., *G.*
Hypholoma fasciculare (Huds.) Fr., *D.*, *G.*
Claudopus variabilis (Pers.) W. G. Sm., on *Epilobium*, *G.*
Polyporus varius Fr., *D.*, squamosus (Huds.) Fr., *G.*, hispidus (Bull.) Fr., *G.*, radiatus (Sow.) Fr., *D.*, caesius (Schrad.) Fr., *G.*
Fomes pomaceus (Pers.) Big. & Guill. on *Prunus spinosa*, *G.*
Ganoderma applanatum (Pers.) Pat., Cromford.
Polystictus versicolor (Linn.) Fr., *D.*, *G.*
Irpex obliquus (Schrad.) Fr., *G.*
Grandinia farinacea (Pers.) Bourd. & Galz., *G.*
Phylacteria terrestris (Ehrh.) Big. & Guill., *G.*
Stereum rugosum (Pers.) Fr., *G.*, hirsutum (Willd.) Fr., *D.*, *G.*
Hymenochaete rubiginosa (Dicks.) Lév., *G.*
Corticium Sambuci (Pers.) Fr., *G.*
Peniophora pallidula Bres., *G.*, byssoidea (Pers.) v. Hoehn. & Litsch., *G.* cinerea (Fr.) Cke, *G.*, quercina (Pers.) Cke. on *Fraxinus*, *D.*
Cyphella capule (Holmsk.) Fr., *G.*
Pistillaria micans (Pers.) Fr., *G.*
Tremella mesenterica (Retz.) Fr., *G.*
Exidia Thuretiana (Lév.) Fr., *G.*
Dacryomyces deliquescens (Bull.) Duby., *G.*

GASTEROMYCETES

Lycoperdon pyriforme (Schaeff.) Pers., *G.*

UREDINALES

Uromyces Valerianae (Schum.) Fuck., *D.*, Alchemillae (Pers.) Lév., *G.*, Ficariae (Schum.) Lév., *G.*, Scillarum (Grev.) Wint., *G.*, Poae Rabenh. on *Poa* and *Ranunculus Ficaria*, *G.*
Puccinia Centaureae Mart., *D.*, *G.*, Carduorum Jacky on *Carduus crispus*, *G.*, obtegens (Link.) Tul., *D.*, *G.*, Lapsanae (Schultz) Fuck., *G.*, Taraxaci Plowr., *D.*, *G.*, Celakovskyana Bub., *G.*, Menthae Pers., Hotel Garden, Saniculae Grev., *D.*, *G.*, Pimpinellae (Str.) Mart., *D.*, Chaerophylli Purt., *D.*, *G.*, pulverulenta Grev., *D.*, *G.*, Violae (Schum.) DC., *D.*, *G.*, Chrysosplenii Grev. on *Chrysosplenium oppositifolium*, *D.*, fusca (Relh.) Wint., *G.*, Lychnidearum Link, *G.*, Caricis (Schum.) Rebent. on *Urtica*, *D.*, Lolii Niels., *G.*, glumarum (Sch.) Eriks. & Henn. on *Brachypodium*, *D.*, sessilis Schneid. on *Phalaris* and *Allium*, *D.*, Winteriana Magn. on *Allium ursinum*, *D.*, Antirrhini Diet. & Holw., Hotel Garden.
Triphragmium Ulmariae (Schum.) Link, *G.*, *D.*
Phragmidium Sanguisorbae (DC.) Schroet., *D.*, mucronatum (Pers.) Schlecht., *D.*, violaceum (Schultz) Wint., *D.*, *G.*
Melampsora Rostrupii Wagn., *D.*, *G.*
Melampsorella Caryophyllacearum (DC.) Schroet. on *Cerastium arvense*, *G.*
Milesia Scolopendrii (Fuck.), Hotel Garden.
Kuehneola albida (Kuehn.) Magn., *G.*

USTILAGINALES

Ustilago violacea (Pers.) Wint., *D.*, *G.*
Entyloma Ranunculi (Bonord.) Schroet., *G.*
Urocystis Anemones (Pers.) Schroet., *G.*

PYRENOMYCETES

Sphaerotheca pannosa (Wallr.) Lév., *G.*
Erysiphe Cichoracearum DC. on *Arctium*, *D.*, Polygoni DC. on *Heracleum*, *D.*
Uncinula Aceris (DC.) Sacc., *D.*
Nectria cinnabarina (Tode) Fr., *MB.*, coccinea (Pers.) Fr. on *Sambucus*, *D.*, *G.*, on *Ulmus*, *G.*, sinopica Fr. on *Hedera*, D., mammoidea Phill. & Plowr. on *Ulmus*, *G.*, Veuillotiana Sacc. & Roum. on *Ilex*, *G.*, episphaeria Fr. on *Diatrype Stigma* on *Crataegus*, *D.*
Chaetomium elatum Kunze, *G.*
Leptospora ovina (Pers.) Fuck. on *Urtica*, *D.*
Melanomma pulvis-pyrius (Pers.) Fuck. on *Crataegus*, *D.*, *G.*, on *Ulmus*, *G.*
Stigmatea Robertiana Fr., *D.*
Didymosphaeria fenestrans (Duby) Wint. on *Epilobium*, *D.*
Leptosphaeria acuta (Moug. & Nestl.) Karst. on *Urtica*, *G.*
Ophiobolus porphyrogonus (Tode) Sacc., *D.*, Bardanae (Fuck.) Rehm on *Heracleum*, *D.*
Quaternaria dissepta (Fr.) Tul., *G.*
Eutypa Acharii Tul., *G.*
Diatrypella verruciformis (Ehrh.) Nitschke on *Corylus*, *D.*, nigroannulata (Grev.) Nitschke on *Corylus*, *D.*, *G.*
Diatrype Stigma (Hoffm.) Fr. 9 on *Crataegus*, *Corylus*, *D.*
Hypoxylon fuscum (Pers.) Fr., *D.*, multiforme Fr., *D.*
Xylaria Hypoxylon (Linn.) Grev., *G.*, polymorpha (Pers.) Grev., *G.*
Scirrhia rimosa (Alb. & Schw.) Fuck., *D.*
Rhopographis filicinus (Fr.) Nitschke.

HYSTERIALES

Lophodermium Pinastri (Schrad.) Chev., *G.*

DISCOMYCETES

Ascomyces alnitorquus (Tul.) Sadeb., *G.*
Calycella citrina (Hedw.) Quél., *D.*
Coryne sarcoides (Jacq.) Tul., *D.*
Orbilia leucostigma Fr., *D.*, xanthostigma Fr., *G.*
Chlorosplenium aeruginosum (Oeder) de Not. (sterile) on *Fraxinus*, *G.*
Helotium herbarum (Pers.) Fr., *D.*
Dasyscypha virginea (Batsch) Fuck., *D.*
Trichoscypha calycina (Schum.) Boud., *G.*
Mollisia cinerea (Batsch) Karst., *G.*
Catinella olivacea (Batsch) Boud., *G.*
Phacidium multivalve (DC.) Kunze & Schm., *G.*
Rhytisma acerinum (Pers.) Fr., *G.*

PHYCOMYCETES

Synchytrium Taraxaci de By. & Woron., *D.*, *G.*, Mercurialis Fuck., *D.*, *G.*
Entomophthora dipterigena Thaxt., *D.*
Plasmopara nivea (Unger) Schroet. on *Aegopodium*, *Angelica*, *Myrrhis*, *D.*, *G.*
Bremia Lactucae Regel on *Centaurea*, *G.*
Peronospora Alsinearum Casp. on *Cerastium*, *G.*, Ficariae Tul., *G.*, sordida Berk. on *Mimulus*, *G.*, parasitica (Pers.) Tul. on *Arabis*, *Sisymbrium*, *D.*

DEUTEROMYCETES

Actinonema Rosae (Lib.) Fr., *MB.*
Phoma acuta Fuck., *D.*, siliquastrum Desm., *G.*, samarum Desm., *D.*
Microphoma Fraxini Delacr., *D.*

Diplodia inquinans West., *G.*
Septoria Bromi Sacc., *D.*, Fraxini West., *D.*, *G.*, Hederae Desm., *G.*, caricola Desm., *G.*
Cercospora Mercurialis Passer., *D.*, *G.*
Botrytis cinerea Pers., *D.*
Ovularia obliqua (Cke.) Oud., *D.*, duplex Sacc., *G.*, primulana Karst., *G.*
Ramularia Colesporii Sacc., *D.*, Scolopendrii Fautr., *MB.*, Urticae Ces., *D.*, lychnicola Cke., *G.*, lactea (Desm.) Sacc., *D.*, Spiraeae Peck, *D.*, *G.*, Tulasnei Sacc., *G.*, Gei (Eliass) Lindr., *D.*, *G.*, Primulae Thüm., *D.*, montana Speg., *D.*, arvensis Sacc., *G.*, calcea (Desm.) Ces., *D.*, *G.*, Ajugae (Niessl) Sacc., *D.*, *G.*, Stachydis (Passer) Massal., *D.*, purpurascens Wint., *D.*, Cirsii Allesch., *G.*, Lampsanae (Desm.) Sacc., *G.*, Taraxaci Karst., *D.*, *G.*
Cladosporium herbarum (Pers.) Link, *D.*
Cladotrichium triseptatum B. & Br., *G.*
Ozonium auricomum Link, *D.*

MYXOMYCETES

By T. Petch

Didymium squamulosum Fr., *D.*
Comatricha nigra Schroet., *D.*, *MB.*
Trichia contorta Rost., *D.*, varia Pers., *G.*
Hemitrichia Karstenii List., *G.*
Perichaena corticalis Rost., *G.*, depressa Libert, *G.*
Lycogala epidendrum Fr., *G.*
Reticularia Lycoperdon Bull., *G.*

THE TOTNES FORAY

23 *to* 28 *September*, 1935

By E. M. WAKEFIELD

The thirty-ninth Autumn Foray and Annual General Meeting was held at Totnes, South Devon, from 23–28 September 1935, with headquarters at the Seymour Hotel. About thirty members and friends took part.

The first expedition, on Tuesday, 24 September, was arranged under the guidance of Mr W. E. Hiley to Mr Kitson's estate at Heatree, on Dartmoor. Unfortunately the day turned out wet and misty, so that little of the moor itself could be seen. The woods were partly pure conifer and partly mixed, and the first wood visited provided a certain amount of shelter, so that vigorous collecting could be carried out. Among many species recorded were *Nolanea icterina*, *Boletus tridentinus*,* *Aleurodiscus amorphus*, and *Empusa apiculata*, the latter reported later by Mr Petch and new to Britain. After lunch a move was made to some higher ground with mixed woods, but conditions were so wet that most people were glad to get back as soon as possible to the cars and make for tea, which was obtained at Manaton. Before leaving members expressed their sincere thanks to Mr Kitson, Junior, who had accompanied them as an additional guide, for the opportunity given to explore these beautiful woods.

Wednesday proved better as regards weather, fine and warm, and was occupied in a most interesting excursion to the estate of Dartington Hall, near Totnes. Here there was considerable variety of ground, including a little pasture and roadside as well as woodland. Almost at once *Puccinia Iridis* was noted on *Iris foetidissima*, which was growing in abundance along a field path. Another noteworthy rust was *Kuehneola albida*, which was common everywhere on bramble. Wood-rotting fungi received some attention: *Ganoderma applanatum* on beech and *Polyporus dryadeus* on oak were among the early finds. Then, later, special attention was drawn to the unusual sight of *Armillaria mellea* fructifications arising about 3 ft. up the trunk of *Pyrus Aucuparia*. Mr Cartwright secured *Coniophorella olivacea*, and Mr Wilkins *Cantharellus amethysteus*. Mr Petch subsequently reported finding *Solenia fasciculata* and *Gonatorrhodiella parasitica* Thaxt., another new British record. At the end of a very pleasant and busy day the party were

* Mr Ramsbottom was shown this species by the late Abbé Bresadola in 1925; it is undoubtedly the same as *Boletus aurantiporus* Howse.

entertained at tea at Dartington Hall, and afterwards heard a most interesting account, given by Dr Slater, Managing Director, of the history, development and aims of this experiment in rural reconstruction. Before leaving they passed a very hearty vote of thanks to the Trustees and staff for their hospitality, and expressed the hope that they might be allowed to repeat the visit at some future time.

The following day, Thursday, good hunting took place in the grounds of Berry Pomeroy Castle, the property of the Duke of Somerset. This proved the richest ground of the week, and many rare species were recorded, among others *Inocybe rhodiola* and *Psalliota xanthoderma* var. *lepiotoides*, which many members thought should be regarded as a species, seeing that it is very different in appearance from the usual *P. xanthoderma*, and in fact strongly suggests a *Lepiota*. *Clavaria aurea*, *Xylaria longipes*, *Nectria Ralfsii*, and *N. sinopica* may also be mentioned. *Aleurodiscus aurantius*, which was found in some quantity on a pile of old branches, has not previously been published as British, but was found some years ago near Newton Abbot by Miss Eyre.

Friday morning was occupied in work at headquarters, but in the afternoon a visit was paid to Seale-Hayne Agricultural College, near Newton Abbot, at the kind invitation of the Principal and staff. Mr Beaumont and Dr Gregory took charge of the party, and after a short account of the history of the college various exhibits illustrating the mycological work there were inspected. A walk round the grounds added a few species to the list, but nothing of any importance. After tea and a very hearty vote of thanks to our hosts the party returned to Totnes.

During the week evening meetings were held as usual. At the Annual General Meeting held on Tuesday, 24 September, the officers for 1936 were elected. Mr F. G. Gould was chosen as President for 1936, with Dr Malcolm Wilson and Mr Cartwright as Vice-Presidents.

The other officers were re-elected. New members of Council, elected to replace three members retiring under the rules, were Miss D. M. Cayley, Dr G. Morgan, and Mr W. C. Moore. Mr Chesters was appointed as delegate to the British Association at Blackpool. The Abbé Bourdot was unanimously elected as an Honorary Member.

After some discussion it was decided to accept the cordial invitation of Irish botanists to visit Killarney for the autumn foray in 1936.

On Wednesday evening the President, Dr Malcolm Wilson, delivered his Presidential Address, on "Some Aspects of Forest Pathology". On Thursday evening Mr Carleton Rea was to have given a paper on the genus *Russula*, but through illness was unable to be present. In his place Miss E. L. Stephens gave a talk on the fungi, particularly Gasteromycetes, of South Africa, and did her best to

persuade members of the British Mycological Society to visit South Africa and help them to name their species. Mr Ramsbottom followed with an account of the International Botanical Congress at Amsterdam, to which Miss Wakefield also added some remarks. At the close of the meeting very hearty votes of thanks were passed to the various landowners whose estates had been visited, and a special vote of thanks to Mr Hiley, manager of the Woodlands Department of Dartington Hall, who had arranged the excursions and obtained the necessary permits.

The following list gives the names of species found during the week, with the exception of a few which have remained as puzzles. For assistance in its compilation the secretary is indebted to all members present, but particularly to Mr Petch, Mr Ramsbottom, Mr Pearson, and Mr Chesters.

Complete List of Species gathered during the Foray

H. = Heatree, Dartmoor; *D.* = Dartington Hall; *B.* = Berry Pomeroy; *S.* = Seale Hayne Agricultural College; *T.* = Totnes neighbourhood.

HYMENOMYCETES

Amanita phalloides (Vaill.) Fr., *D.*, porphyria (A. & S.) Fr., *D.*, mappa (Batsch) Fr., *D.*, pantherina (DC.) Fr., *B.*, spissa Fr., *D.*, rubescens (Pers.) Fr., *H.*, *B.*

Amanitopsis vaginata (Bull.) Roze, *D.*, *B.*, fulva (Schaeff.) W. G. Sm., *D.*, strangulata (Fr.) Roze, *B.*

Lepiota procera (Scop.) Fr., *D.*, rhacodes (Vitt.) Fr., *B.*, *S.*, excoriata (Schaeff.) Fr., *D.*, *S.*, acutesquamosa (Weinm.) Fr., *B.*, *D.*, felina (Pers.) Fr., *B.*, cristata (A. & S.) Fr., *H.*, *D.*, *B.*, castanea Quél., *B.*, sistrata Fr., *D.*, *B.*

Armillaria mellea (Vahl) Fr., *H.*, *D.*, *B.*, mucida (Schrad.) Fr., *H.*, *D.*, *B.*

Tricholoma albobrunneum (Pers.) Fr., *D.*, rutilans (Schaeff.) Fr., *H.*, *D.*, *B.*, terreum (Schaeff.) Fr., *B.*, cartilagineum Fr. non Bull., *H.*, cuneifolium Fr., H., saponaceum Fr., *D.*, nudum (Bull.) Fr., *B.*, polioleucum Fr., *B.*, sordidum (Schum.) Fr., *B.*

Clitocybe nebularis (Batsch) Fr., H., clavipes (Pers.) Fr., *H.*, aurantiaca (Wulf.) Studer, *H.*, rivulosa (Pers.) Fr., *S.*, cerussata Fr., *D.*, infundibuliformis (Schaeff.) Fr., *D.*, *B.*, vibecina Fr., *B.*

Laccaria laccata (Scop.) B. & Br., *H.*, *D.*, *B.*, and var. amethystina (Vaill.) B. & Br., *H.*, *D.*

Collybia radicata (Relh.) Berk., *H.*, *D.*, *B.*, platyphylla (Pers.) Fr., *H.*, *D.*, *B.*, fusipes (Bull.) Berk., *D.*, maculata (A. & S.) Fr., *H.*, distorta Fr., *H.*, butyracea (Bull.) Fr., *H.*, tuberosa (Bull.) Fr., *H.*

Mycena pelianthina Fr., *H.*, *B.*, rubromarginata Fr., *H.*, *D.*, pura (Pers.) Fr., *H.*, *B.*, Adonis (Bull.) Fr., *B.*, galericulata (Scop.) Fr., *B.*, polygramma (Bull.) Fr., *B.*, ammoniaca Fr., *H.*, metata Fr., *H.*, amicta Fr., *D.*, Iris Berk., *H.*, vitilis Fr., *D.*, *B.*, haematopus (Pers.) Fr., *H.*, *B.*, sanguinolenta (A. & S.) Fr., *H.*, *D.*, *B.*, galopus (Pers.) Fr., *H.*, *B.*, and var. alba Fl. Dan., *H.*, *B.*, epipterygia (Scop.) Fr., *B.*

Omphalia fibula (Bull.) Fr., *H.*, *D.*

Hygrophorus eburneus (Bull.) Fr., *B.*, cossus (Sow.) Fr., *B.*, coccineus (Schaeff.) Fr., *D.*, *B.*, conicus (Scop.) Fr., *S.*

Lactarius insulsus Fr., *D.*, blennius Fr., *H.*, chrysorheus Fr., *H.*, deliciosus (Linn.) Fr., *D.*, pallidus (Pers.) Fr., *B.*, quietus Fr., *H.*, *B.*, aurantiacus (Fl. Dan.) Fr., *D.*, rufus (Scop.) Fr., *H.*, fuliginosus Fr., *D.*, serifluus (DC.) Fr., *H.*, mitissimus Fr., *H.*, *D.*, subdulcis (Pers.) Fr., *H.*, *B.*

Russula chloroides (Krombh.) Bres., *H.*, lepida Fr., *D.*, cyanoxantha (Schaeff.) Fr., *H.*, *B.*, fellea Fr., *H.*, fragilis (Pers.) Fr., *D.*, luteotacta Rea, *D.*, atropurpurea (Krombh.) Maire, *D.*, xerampelina (Schaeff.) Fr., *D.*, lutea (Huds.) Fr., *B.*, and var. armeniaca (Cooke) Rea, *D.*, *B.*, paludosa Britz., *D.*, pectinata (Bull.) Fr., *B.*

Cantharellus cibarius Fr., *D.*, *B.*, amethysteus Quél., *D.*, lutescens (Pers.) Fr., *D.*

Marasmius peronatus (Bolt.) Fr., *H.*, oreades (Bolt.) Fr., *H.*, *S.*, globularis Fr., *B.*, erythropus (Pers.) Fr., *B.*, hariolorum (DC.) Quél., *H.*, *B.*, dryophilus (Bull.) Karst., *H.*, *B.*

Androsaceus rotula (Scop.) Pat., *D.*, androsaceus (Linn.) Pat., *H.*

Panus torulosus (Pers.) Fr., *D.*, *B.*, stipticus (Bull.) Fr., *D.*, *B.*

Lentinus cochleatus (Pers.) Fr., *D.*, *B.*

Volvaria speciosa Fr., *D.*

Pluteus cervinus (Schaeff.) Fr., *D.*, salicinus (Pers.) Fr., *H.*, nanus (Pers.) Fr., *D.*

Entoloma rhodopolium Fr., *D.*, *B.*, nidorosum Fr., *D.*

Nolanea proletaria Fr., *H.*, *B.*, papillata Bres., *D.*, cetrata (Fr.) Schroet., *H.*, icterina Fr., *H.*

Leptonia sericella (Fr.) Quél., *H.*

Clitopilus prunulus (Scop.) Fr., *B.*

Claudopus variabilis (Pers.) W. G. Sm., *H.*, *D.*, *B.*

Paxillus involutus (Batsch) Fr., *H.*

Pholiota erebia Fr., *H.*, *B.*, squarrosa (Muell.) Fr., *T.*, *D.*, adiposa Fr., *B.*, mutabilis (Schaeff.) Fr., *H.*

Inocybe tomentosa (Jungh.) Quél., *H.*, *B.*, geophylla (Sow.) Fr., *H.*, *B.*, and var. lilacina Fr., *D.*, descissa Fr., *H.*, Godeyi Gill., *H.*, Cookei Bres., *H.*, *D.*, *B.*, rhodiola Bres., *B.*, maculata Boud., *D.*, obscura (Pers.) Fr., *D.*

Astrosporina umbrina (Bres.) Rea., *D.*, petiginosa (Fr.) Rea, *D.*

Hebeloma glutinosum (Lindgr.) Fr., *H.*, mesophaeum Fr., *B.*, crustuliniforme (Bull.) Fr., *H.*, *D.*, longicaudum (Pers.) Fr., *B.*

Galera tenera (Schaeff.) Fr., *H.*, hypnorum (Schrank) Fr., *H.*, *B.*

Naucoria Cucumis (Pers.) Fr., *B.*, melinoides Fr., *B.*, escharoides Fr., *H.*

Tubaria furfuracea (Pers.) W. G. Sm., *H.*, *D.*

Flammula sapinea Fr., *H.*, ochrochlora Fr., *D.*

Cortinarius (Phlegmacium) cyanopus (Secr.) Fr., *B.*, infractus (Pers.) Fr., *B.*, caerulescens Fr., *D.* (Myxacium), elatior Fr., *D.*, (Inoloma) alboviolaceus (Pers.) Fr., *H.*, *B.*, (Dermocybe) cinnamomeus (Linn.) Fr., *H.*, (Telamonia) torvus Fr., *B.*, hinnuleus (Sow.) Fr., *D.*, hemitrichus Fr., *B.*, (Hydrocybe) duracinus Fr., *D.*, castaneus (Bull.) Fr., *B.*, decipiens (Pers.) Fr., *D.*, acutus (Pers.) Fr., *B.*, *S.*

Crepidotus mollis (Schaeff.) Fr., *T.*, *D.*, *B.*, calolepis Fr., *B.*

Bolbitius fragilis (Linn.) Fr., *D.*, *S.*

Psalliota xanthoderma Genev., var. lepiotoides R. Maire, *B.*, campestris (Linn.) Fr., *S.*, sylvicola (Vitt.) Fr., *D.*, *B.*, amethystina Quél., *D.*, sylvatica (Schaeff.) Fr., *B.*, arvensis (Schaeff.) Fr., *H.*

Stropharia semiglobata (Batsch) Fr., *H.*, aeruginosa (Curt.) Fr., *B.*

Hypholoma fasciculare (Huds.) Fr., *H.*, *B.*, velutinum (Pers.) Fr., *H.*, *D.*, Candolleanum Fr., *B.*, hydrophilum (Bull.) Fr., *D.*, *B.*

Psathyra fibrillosa (Pers.) Fr., *H.*

Psathyrella gracilis Fr., *B.*, atomata Fr., *H.*, *B.*, disseminata (Pers.) Fr., *D.*, *B.*

Panaeolus sphinctrinus Fr., *H.*, *S.*, campanulatus (Linn.) Fr., *H.*

Coprinus comatus (Fl. Dan.) Fr., *D.*, atramentarius (Bull.) Fr., *D.*, cinereus (Schaeff.) Cke, *H.*, *B.*, micaceus (Bull.) Fr., *H.*, *B.*, plicatilis (Curt.) Fr., *S.*

Gomphidius viscidus (Linn.) Fr., *B.*

Boletus elegans (Schum.) Fr., *H.*, viscidus (Linn.) Fr., *H.*, badius Fr., *H.*, bovinus (Linn.) Fr., *H.*, chrysenteron (Bull.) Fr., *H.*, *D.*, *B.*, subtomentosus (Linn.) Fr., *H.*, *B.*, versicolor Rostk., *B.*, impolitus Fr., *D.*, edulis (Bull.) Fr., *H.*, *B.*, calopus Fr., *D.*, erythropus Fr. non Pers., *H.*, purpureus Fr., *D.*, tridentinus Bres., *H.*, scaber (Bull.) Fr., *D.*, castaneus (Bull.) Quél., *B.*, porphyrosporus Fr., *B.*

Polyporus squamosus (Huds.) Fr., *D.*, *B.*, Schweinitzii Fr., *B.*, giganteus (Pers.) Fr. on hawthorn, *H.*, on beech, *D.*, dryadeus (Pers.) Fr., *D.*, hispidus (Bull.) Fr., *B.*, radiatus (Sow.) Fr., *D.*, adustus (Willd.) Fr., *H.*, *D.*, *T.*, fragilis Fr., *B.*, caesius (Schrad.) Fr., *H.*, tephroleucus Fr., *B.*
Fomes Ribis (Schum.) Fr. on *Euonymus*, *B.*, ferruginosus (Schrad.) Mass., *B.*, ulmarius (Sow.) Fr., *D.*, fraxineus (Bull.) Fr., on *Ulmus*, *T.*, annosus Fr., *H.*, *B.*
Ganoderma applanatum (Pers.) Pat., *D.*
Poria hymenocystis B. & Br., *H.*
Polystictus versicolor (Linn.) Fr., *D.*, *B.*, abietinus (Dicks.) Fr., *B.*
Irpex obliquus (Schrad.) Fr., *D.*, *B.*
Lenzites betulina (Linn.) Fr., *B.*
Trametes gibbosa (Pers.) Fr., *D.*, *B.*
Daedalea biennis (Bull.) Quél., *D.*, quercina (Linn.) Fr., *D.*
Merulius tremellosus (Schrad.) Fr., *H.*, corium (Pers.) Fr., *D.*, rufus (Pers.) Fr., *H.*, *D.*
Phlebia merismoides Fr., *H.*
Coniophorella olivacea (Fr.) Karst., *D.*
Hydnum repandum (Linn.) Fr., var rufescens (Pers.) Fr., *D.*
Mycoleptodon ochraceum (Pers.) Pat., *B.*
Acia uda (Fr.) Bourd. & Galz., *D.*
Tomentella mucidula (Karst.) v. Hoehn. & Litsch. (=Hypochnus roseogriseus Wakef. & Pears.), *H.*
Stereum spadiceum Fr., *H.*, *D.*, rugosum (Pers.) Fr., *H.*, hirsutum (Willd.) Fr., *D.*, *B.*, purpureum (Pers.) Fr., *D.*
Hymenochaete rubiginosa (Dicks.) Lév., *B.*, corrugata (Fr.) Lév. on *Crataegus*, *H.*, *D.*
Aleurodiscus amorphus (Pers.) Rabenh., *H.*, aurantius (Pers.) Schroet., *B.*
Corticium botryosum Bres., *H.*, confluens Fr., *H.*, porosum B. & C., *H.*
Vuilleminia comedens (Nees) R. Maire, *D.*
Peniophora Molleriana (Bres.) Sacc. on *Quercus*, *B.*, quercina (Pers.) Cke, *D.*, cinerea (Fr.) Cke, *B.*
Solenia fasciculata Pers., *D.*
Clavaria cristata (Holmsk.) Fr., *H.*, *D.*, cinerea (Bull.) Fr., *B.*, rugosa (Bull.) Fr., *H.*, aurea (Schaeff.) Fr., *B.*, stricta (Pers.) Fr., *B.*, vermicularis Fr., *H.*
Pistillaria puberula Berk., *D.*
Auricularia mesenterica (Dicks.) Fr., *D.*, *T.*, auricula-Judae (Linn.) Schroet., *B.*
Tremella mesenterica (Retz.) Fr., *D.*
Exidia nucleata (Schw.) Rea, *D.*, Thuretiana (Lév.) Fr., *B.*
Sebacina incrustans (Pers.) Tul., *B.*
Calocera viscosa (Pers.) Fr., *H.*, *B.*, cornea (Batsch) Fr., *H.*, *B.*

GASTEROMYCETES

Cynophallus caninus (Huds.) Fr., *H.*
Phallus impudicus (Linn.) Pers., *H.*
Lycoperdon saccatum (Vahl) Fr., *D.*, *B.*, depressum Bon., *S.*, perlatum Pers., *H.*, *D.*, pyriforme (Schaeff.) Pers., *D.*, *B.*
Bovistea plumbea Fr., *S.*
Cyathus striatus (Huds.) Pers., *D.*
Scleroderma aurantium Pers., *H.*, *D.*, *B.*
Sphaerobolus stellatus (Tode) Pers., *D.*

UREDINALES

Uromyces Rumicis (Schum.) Wint., *B.*, Poae Rabenh., *B.*
Puccinia Violae (Schum.) DC., *H.*, Lychnidearum Link, *H.*, *D.*, Circaeae Pers., *D.*, *B.*, Umbilici Guép. *H.*, *B.*, Angelicae (Schum.) Fuck., *B.*, Conii (Str.) Fuck., *B.*, Saniculae Grev., *B.*, obtegens (Link) Tul., *D.*, Cirsii Lasch, *B.*, Lampsanae (Schultz) Fuck., *B.*, Primulae (DC.) Duby, *B.*, Veronicae Schroet., *D.*, Antirrhini Diet. & Holw., *T.*, Glechomatis DC., *D.*, Menthae Pers., *B.*, Iridis (DC.) Wallr., on *Iris foetidissima*, *D.*, *B.*, oblongata (Link) Wint., *D.*, sessilis Schneid., *B.*
Triphragmium Ulmariae (Schum.) Link, *B.*
Phragmidium Potentillae (Pers.) Wint., *D.*, Sanguisorbae (DC.) Schroet., *D.*, Rubi (Pers.) Wint., *D.*, violaceum (Schultz) Wint., *H.*, *D.*, *B.*, Rubi-idaei (Pers.) Karst., *H.*
Kuehneola albida (Kuehn.) Magn., *D.*
Coleosporium Senecionis (Pers.) Fr., *T.*, Tussilaginis (Pers.) Kleb., *B.*
Pucciniastrum Abieti-Chamaenerii Kleb., *D.*, Agrimoniae (DC.) Tranzsch., *D.*, *B.*, Circaeae (Schum.) Schroet., *D.*
Melampsora Hypericorum (DC.) Schroet., *D.*, Euphorbiae Cast., *T.*
Milesina Kriegeriana P. Magn. on *Lastraea dilatata*, *H.*, on *Polypodium*, *H.*

USTILAGINALES

Ustilago violacea (Pers.) Wint., *D.*
Sphacelotheca Hydropiperidis (Schum.) de Bary, *D.*, *B.*
Urocystis Anemones (Pers.) Schroet. on *Ranunculus repens*, *B.*

PYRENOMYCETES

Erysiphe Cichoracearum DC. on *Arctium*, *H.*, *D.*, on *Plantago*, *D.*, Polygoni DC. on *Delphinium*, *T.*, on *Heracleum*, *D.*, tortilis (Wallr.) Fr. on *Cornus*, *B.*
Uncinula Aceris (DC.) Sacc., *H.*
Phyllactinia corylea (DC.) Karst. on *Fraxinus*, *H.*, *B.*
Melanospora parasitica Tul. on *Beauveria Bassiana*, *D.*
Nectria cinnabarina (Tode) Fr., on *Fagus*, *H.*, *B.*, *D.*, coccinea (Pers.) Fr., *D.*, *B.*, sinopica Fr. on *Hedera*, *B.*, Ralfsii B. & Br. on *Acer*, *B.*, galligena Bres. on *Quercus*, *D.*, mammoidea Phill. & Plowr., *D.*
Sphaerostilbe aurantiaca Tul. on *Ulmus*, *D.*
Hypomyces aurantius (Pers.) Fuck. on *Polystictus versicolor*, *D.*, on *Stereum hirsutum*, *B.*
Apiocrea chrysosperma (Tul.) Syd. on *Boletus*, *D.*
Claviceps purpurea (Fr.) Tul. on *Dactylis glomerata*, *D.*
Cordyceps militaris (Linn.) Fr., *D.*
Trichosphaeria minima (Fuck.) Wint. on *Fraxinus*, *D.*
Leptospora ovina (Pers.) Fuck., *D.*
Melanomma fusciduluni Sacc. on *Fagus*, *H.*, pulvis-pyrius (Pers.) Fuck. on *Fagus*, *Corylus* and *Tilia*, *H.*
Stigmatea Robertiani Fr., *H.*
Diaporthe taleola (Fr.) Sacc. on *Quercus*, *D.*, leiphaemia (Fr.) Sacc. on *Quercus*, *D.*, eres Nitschke on *Hedera*, *D.*
Cryptosphaeria eunomia (Fr.) Fuck. on *Fraxinus*, *D.*
Eutypella stellulata (Fr.) Sacc. on *Ulmus*, *D.*
Quaternaria quaternata (Pers.) Schroet. on *Fagus*, *H.*, *D.*, dissepta (Fr.) Tul. on *Ulmus*, *D.*
Diatrypella verruciformis (Ehrh.) Nitschke on *Fagus*, *H.*, quercina (Pers.) Nitschke on *Quercus*, *D.*
Diatrype Stigma (Hoffm.) Fr. on *Fagus*, *H.*, *D.*
Ustulina vulgaris Tul., *B.*
Hypoxylon coccineum Bull. on *Fagus*, *D.*, *B.*, fuscum (Pers.) Fr., *B.*, multiforme Fr. on *Corylus*, *H.*, rubiginosum (Pers.) Fr. on *Fagus*, *D.*

Nummularia lutea (A. & S.) Nitschke, *D.*
Daldinia concentrica (Bolt.) Ces. & de Not., *B.*
Xylaria Hypoxylon (Linn.) Grev., *H.*, *B.*, longipes Nitschke, *B.*
Rhopographus filicinus (Fr.) Nitschke, *H.*

DISCOMYCETES

Peziza aurantia Pers., *D.*
Lachnea hemisphaerica (Wigg.) Gill., *B.*
Ciliaria scutellata (Linn.) Quél., *H.*
Humaria carbonigena Berk., *D.*
Cudoniella acicularis (Bull.) Schroet., *D.*
Calycella citrina (Hedw.) Quél., *B.*
Bulgaria inquinans (Pers.) Fr., *H.*, *D.* (on *Fagus*).
Chlorosplenium aeruginosum (Oeder) de Not., *D.*, *B.*
Dasyscypha calycina (Schum.) Fuck., *H.*
Hyaloscypha hyalina (Pers.) Boud., *D.*
Mollisia cinerea (Batsch) Karst., *D.*
Phacidium multivalve (DC.) Kunze & Schm., *B.*
Pseudopeziza Trifolii (Biv.-Bern.) Fuck., *D.*
Fabraea Ranunculi (Fr.) Karst., *B.*
Rhytisma acerinum (Pers.) Fr., *H.*, *D.*

HYSTERIALES

Lophodermium Pinastri (Schrad.) Chev., *H.*, Rhododendri Ces., *H.*

PHYCOMYCETES

Entomophthora americana Thaxt. on fly, *D.*, sphaerosperma Fres. on fly, *D.*, dipterigena Thaxt. on flies, *H.*, echinospora Thaxt. on fly, *D.*
Empusa apiculata Thaxt. on flies and leaf-hopper, *H.*
Spinellus fusiger (Link) v. Tiegh., *D.*

DEUTEROMYCETES

Phomopsis oblonga Trav. on *Ulmus*, *H.*, *D.*
Actinonema Rosae (Lib.) Fr., *T.*
Septoria Violae West., *D.*, Rubi West., *D.*, *B.*
Monilia aurea Pers., *H.*, *B.*
Microstroma album (Desm.) Sacc. on *Quercus*, *H.*
Ovularia obliqua (Cke) Oud., *H.*
Sepedonium chrysospermum (Bull.) Fr., *H.*, *B.*
Rhinotrichum repens Preuss, *D.*, *B.*
Beauveria Bassiana (Berk.) Vuill. on weevil, *H.*, on bee, *D.*
Trichoderma viride (Pers.) Fr., *D.*
Gliocladium penicillioides Corda on *Dictydium cancellatum*, *H.*
Cephalosporium coccorum Petch on mealy bug, *T.*, muscarium Petch on fly, *D.*
Gonatorrhodiella parasitica Thaxt. on *Hypomyces aurantius*, *D.*
Ramularia sambucina Sacc., *H.*, Primulae Thuem., *H.*
Polythrincium Trifolii Kunze, *D.*
Cladosporium herbarum (Pers.) Link on flies, *D.*
Cercospora Mercurialis Pass., *D.*, *B.*
Heterosporium gracile Sacc. on *Iris*, *D.*
Isaria farinosa (Holmsk.) Fr. on pupae, *H.*, *B.*
Hymenostilbe arachnophila (Ditm.) Petch on spider, *B.*
Gibellula aranearum (Schw.) Syd. on spider, *B.*

MYCETOZOA

By Miss G. Lister

Ceratiomyxa fruticulosa Macbr., *H.*, *D.*, *B.*
Badhamia capsulifera Berk., *D.*
Physarum nutans Pers., *H.*, *D.*, *B.*, viride Pers., *B.*
Craterium minutum Fr., *B.*, leucocephalum Ditm., *B.*
Leocarpus fragilis Rost., *B.*
Didymium difforme Duby, *D.*, melanospermum Macbr. var. minus Lister, *B.*, nigripes Fr., *B.*, and var. xanthopus Lister, *D.*, *B.*, squamulosum Fr., *B.*, dubium Rost., *B.*
Stemonitis fusca Roth, *D.*, *B.*, ferruginea Ehrenb., *H.*
Comatricha nigra Schroet., *H.*, *D.*, *B.*, laxa Rost., *H.*, typhoides Rost., *D.*
Lamproderma scintillans Morgan, *B.*
Cribraria vulgaris Schrad., *B.*
Dictydium cancellatum Macbr., *H.*, *B.*
Tubifera ferruginosa Gmel., *B.*
Lycogala epidendrum Fr., *H.*, *D.*
Trichia persimilis Karst., *D.*, varia Pers., *B.*, contorta Rost., *B.*, decipiens Macbr., *H.*, *D.*, *B.*, Botrytis Pers., *H.*
Hemitrichia vesparium Macbr., *D.*
Arcyria cinerea Pers., *D.*, *B.*, denudata Wettst., *H.*, *D.*, *B.*, incarnata Pers., *H.*, *D.*

LICHENS OF THE TOTNES DISTRICT

By W. Watson

When Totnes was selected as the venue of the 1935 Foray visions of Dartmoor Tors and expectations of seeing species of *Gyrophora*, *Umblicaria*, *Alectoria*, *Stereocaulon* and other lichens of the subalpine rocks were conjured up but Pluvius was too liberal in his welcome. The only official excursion on which the Tors could be visited was the Manaton one and the day was so wet that serious lichenological work was not possible. This loss was to some extent compensated for by a visit with Mr Knight to Lydford but the compensation was far from complete, as is evident by the absence from the following list of the above-mentioned plants and also of such common lichens of the Dartmoor uplands as *Lecanora polytropa*, *Lecidea Dicksonii*, *Cladina sylvatica*, *Cladonia uncialis* and *Baeomyces rufus*.

The Berry Pomeroy woods yielded a fair number of corticicolous species and on a limestone wall at Dartington many interesting species characteristic of such a habitat were found. A *Verrucaria* which has not yet been described was found and a number of other plants new to Devon. Mr Lamb succeeded in finding *Bacidia prasinoides*, another addition from the Kerry flora for south-west England.

Some lichens were so frequent and well distributed that it is needless to specify definite localities for them. These are *Evernia prunastri*, *Parmelia perlata*, *P. caperata* and its forms *sorediosa* and *saxicola*, *P. fuliginosa* and its var. *laetevirens*, *P. saxatilis* and its form *furfuracea*, *P. subaurifera*, *P. conspersa*, *Hypogymnia physodes* and its form *labrosa*,

Lecanora chlarona, *L. rugosa*, *L. allophana*, *L. atra*, *Lecidea parasema* and its var. *flavens*, *L. rivulosa*, *L. contigua*, *Pertusaria faginea*, *P. pertusa*, *P. dealbata*, *P. leioplaca*, *Ramalina farinacea*, *R. fastigiata*, *Placodium callopismum* var. *plicatum* (=*P. flavescens*), *Candelariella vitellina*, *Physcia hispida*, *Cladonia pyxidata*, *C. fimbriata* var. *subulata*, *Peltigera canina*, *P. rufescens*, *P. polydactyla*, *Opegrapha atra*, *O. vulgata*, *Graphis elegans* and *G. scripta*.

In the following list: *B.* = Berry Pomeroy. *D.* = Dartington. *H.* = Holne. *L.* = Lydford. *M.* = Manaton. *T.* = Totnes. An asterisk denotes that the plant has not previously been recorded for Devon.

Usnea florida (L.) Web., *B.*, *L.*, *M.*
var. hirta (L.) Ach., *B.*, *M.*
U. ceratina Ach., *B.*, *H.*, *L.*, *M.*
*form incurviscens Arn., *M.*
U. dasypoga (Ach.) Nyl., *B.*, *D.*
*U. barbata (L.) Hoff., emend. Motyka, *B.*, *M.*
U. articulata (L.) Hoff., *D.*
Evernia furfuracea (L.) Mann., *M.*
*form scobicina (Ach.) Cromb., *M.*
Parmelia prolixa (Ach.) Carr., *M.*
var. isidiostyla Nyl., *M.*
P. revoluta Flk., *D.*, *H.*
P. tiliacea (Hoff.) Ach., *H.*
P. sulcata Tayl., *D.*
P. dubia (Wulf.) Schaer., *D.* On rocks, Dartmouth and Strete
P. omphalodes (L.) Ach., *L.*, *M.*, Dartmeet
Hypogymnia physodes (L.) Nyl.
var. tubulosa (Schaer.) Wats., *M.*
Platysma glaucum (L.) Nyl., *B.*, *D.*, *M.*
var. fallax (Web.) Nyl., *D.*, *M.*
Lecanora albescens (Hoff.) Flk. (=L. galactina Ach.), *L.*, *T.*
L. dispersa (Pers.) Roehl., *T.*
L. Hageni Ach., *M.*, *T.*
L. subfusca (L.) Ach., *B.*, *M.*
L. campestris (Schaer.) Hue, *M.*, *T.*
L. intumescens (Reb.) Krb., *D.*, *H.*, *L.*
L. pallida (Schreb.) Schaer., *H.*
L. carpinea (L.) Wain., *B.*, *D.*
L. expallens (Pers.) Ach., *D.*, *M.*
L. varia (Ehrh.) Ach., *M.*, *T.*
L. symmicta Ach., *H.*, *L.*
var. sepincola (Ach.) Nyl., *L.*
L. symmictera Nyl., *D.*, *H.*, *L.*
Ochrolechia parella (L.) Arn., *D.*, *L.*, *M.*
*form porinoides (Ehrh.) Zahl., *D.*
Aspicilia calcarea (L.) Krb., *B.*, *D.*
A. lacustris (With.) Th. Fr., *H.*
Biatora lucida (Ach.) Fr., *L.*
B. coarctata (Sm.) Th. Fr., *L.*, *M.*
Lecidea macrocarpa (DC.) Steud., *M.*
L. sorediza Nyl., *M.*
*L. cinereoatra Ach., *M.*
*Pertusaria pertusa (L.) Tuck.
var. leiotera (Nyl.) Zahl., *M.*
P. dealbata (Ach.) Cromb.
form corallina (L.) Cromb., *M.*
P. multipuncta (Turn.) Nyl., *D.*
P. globulifera (Turn.) Nyl., *D.*
P. Wulfenii DC., *B.*, *D.*
Biatorella simplex (Dav.) B. & R., Strete
Ramalina calicaris (L.) Fr., *B.*, *D.*
R. fraxinea (L.) Ach., *D.*
Solenopsora holophaea (Mont.) samp., Dartmouth
Biatorina erysiboides (Nyl.) Th. Fr., *D.*, *L.*
B. lenticularis (Ach.) Krb., *D.*
B. Lightfootii (Sm.) Mudd, *B.*, *H.*
Toninia aromatica (Sm.) Mass., Dartmouth
Bilimbia sabuletorum (Flk.) Arn., *D.*, Kingsbridge
Bacidia luteola (Schrad.) Mudd, *B.*
B. phacodes Krb., *L.*
B. effusa (Sm.) Arn., *L.*
*B. prasinoides (Nyl.) Oliv., *D.*
Leciographa parasitica (Flk.) Mass., *D.*
Xanthoria parietina (L.) Th. Fr., *T.*
var. ectanea (Ach.) Oliv., Dartmouth
Placodium murorum (Hoff.) DC., *T.*
P. xantholytum Nyl., *D.*
Callopisma citrinum (Hoff.) Mass., *T.*
C. ochraceum (Schaer.) Mass., *D.*
C. aurantiacum (Lightf.) Mass. var. flavovirescens (Wulf.) Krb., *D.*
C. caesiorufum (Ach.) Wats., Dartmouth
C. ferrugineum (Huds.) Mudd, var. festivum (Ach.) Mudd, *L.*

C. rupestre (Scop.) Wats. var. calvum (Dicks.) Wats., *L.*
Pseudophyscia fusca (Huds.) Wats., Slapton, Dartmouth
Physcia aipolia (Ach.) Nyl., *T.*
P. virella (Ach.) Lynge, *T.*
Diploicia canescens (Dicks.) Mass., *D.*
Buellia myriocarpa (DC.) Mudd, *D.*
B. stellulata (Tayl.) Mudd, Strete
*B. verruculosa (Borr.) Mudd, Strete
B. disciformis (Fr.) Mudd, *H.*
*B. confervoides Kremp., Strete
Rhizocarpon geographicum (L.) DC., *M.*, Dartmeet
*R. viridiatrum (Flk.) Krb., *L.*, *M.*
R. petraeum (Wulf.) Mass., *L.*, *M.*
Thelotrema lepadinum Ach., *B.*
Phlyctis agelaea (Ach.) Krb., *B.*, *D.*
Diploschistes scruposus (L.) Norm., Dartmeet
Cladonia ochrochlora Flk., *M.*
C. cervicornis (Ach.) Schaer. var subcervicornis Wain. *M.*
C. furcata (Huds.) Schrad., *M.*
C. squamosa Hoff., *L.*
C. rangiformis Hoff., *M.*
C. flabelliformis (Flk.) Wain., *M.*
C. macilenta (Ehrh.) Hoff., *B.*
C. coccifera (L.) Willd., *D.*, *M.*
Coenogonium ebeneum (Dillw.) A. L. Sm., *M.*
Crocynia lanuginosa (Ach.) Hue, *T.*
Lobaria pulmonaria (L.) Hoff., *B.*
L. laetevirens (Light.) Zahl., *B.*
Stictina fuliginosa (Dicks.) Nyl., *L.*
S. limbata (Sm.) Nyl., *L.*
S. Dufourii (Del.) Nyl., *L.*
Peltigera rufescens (Weis.) Humb. var. praetextata (Flk.) Nyl., *M.*
*var. lepidophora (Nyl.) Wain., *D.*
P. polydactyla (Neck.) Hoffm. var. hymenina (Ach.) Nyl., *B.*, *M.*
P. horizontalis (L.) Hoff., *L.*, *M.*
P. scutata (Dicks.) Krb., *L.*
Pannaria rubiginosa (Thunb.) Del. var. conoplea (Pers.) Krb., *L.*
Placynthium nigrum (Huds.) Gray, *D.*
Parmeliella corallinoides (Hoff.) Zahl., *L.*
Leptogium sinuatum (Huds.) Mass., *D.*
var. scotinum (Ach.) Krb., Dartmouth
L. lacerum (Lilj.) Gray, *B.*, *D.*, *L.*
L. ruginosum (Duf.) Nyl., Dartmouth
L. tremelloides (L.) Gray, Dartmouth
L. microscopicum Nyl., *L.*
L. plicatile (Ach.) Th. Fr., *L.*
Collema pulposum (Bern.) Ach., *D.*, *L.*
C. granosum (Scop.) Schaer., *D.*
C. granuliferum Nyl., *D.*
C. cheileum Ach., *D.*, *L.*
*form graniforme Harm., *D.*
Ephebeia hispidula (Ach.) Nyl., *H* (c. fr.).
Arthonia gregaria (Weig.) Krb., *D.*
*var. astroidea (Leight.) Mudd, *L.*
*var cuspidans (Nyl.) Wats., *L.*
A. lurida Ach., *B.*
*A. aspersella Leight., *B.*, *H.*
A. radiata (Pers.) Ach., *B.*
var. Swartziana (Ach.) Sydow, *B.*, *L.*
(4*) form parallela (Mudd) Leight., Okehampton
Opegrapha atra Pers. var. denigrata (Ach.) Schaer., *D.*, Okehampton
O. betulina Sm., *B.*
O. calcarea Turn., *M.*
O. saxicola Ach., *D.*
var. Decandollei Stiz., *D.*
O. varia Pers., *D.*
Enterographa crassa (Del.) Fée, *B.*, *D.*
E. Hutchinsiae (Leight.) Krb., *B.*
Graphis scripta (L.) Ach. var. serpentina (Ach.) Nyl., *D.*
Phaeographis dendritica (Ach.) Mull., *B.*, *D.*
Graphina anguina (Mont.) Mull., *B.*
Calicium hyperellum Ach., *D.*
*Cyphelium stigonellum (Ach.) Zahl., *M.*
Coniocybe furfuracea (L.) Ach., *L.*, *M.*
Stenocybe byssacea (Fr.) Nyl., *B.*
Sphaerophorus globosus (Huds.) Wain., *M.*
form congestus (Lamy) A. L. Sm., *M.*
S. fragilis (L.) Pers., *M.*
Dermatocarpon miniatum (L.) Th. Fr., *D.*
Verrucaria maura Wahl., Slapton, Dartmouth
V. viridula (Schrad.) Ach., *D.*
V. maculiformis Kremp., *B.*
V. papillosa Ach., *L.*
V. glaucina Ach., *D.*
V. rupestris Schrad., *D.*
V. nigrescens Pers., *D.*, *T.*
V. muralis Ach., *D.*
V. sphinctrina (Duf.) Nyl., *D.*
*V. calciseda DC. emend Stein, *D.*
*form calcivora Mass., *D.*
Acrocordia epipolaea (Borr.) A. L. Sm., *D.*

Arthopyrenia punctiformis (Pers.) Arn., *B.*, *H.*
A. fallax (Nyl.) Arn., *H.*
A. submicans (Nyl.) A. L. Sm., *B.*, M.
Porina carpinea (Pers.) Zahl., *H.*, *L.*
P. chlorotica (Ach.) Wain., *D.*, *M.*
*P. affinis (Mass.) Zahl., *L.*
*P. leptalea (D. & M.) A. L. Sm., *H.*
Pyrenula nitida (Weig.) Ach., *B.*, *D.*
form geographica (Cromb.) Wats., *B.*
var. nitidella (Flk.) Mudd, *B.*, *D.*

Owing to the restricted conception of some of the species of *Usnea* by Dr Motyka of Poland, it seems necessary to mention that the Dartington *U. dasypoga* scarcely agrees with the species as restricted by him, though it agrees with *U. dasypoga* of most authors. On the other hand, *U. florida* var. *hirta*, a name which has been very loosely applied in this country, agrees with both Stirton's and Motyka's conceptions of *hirta*.

NEW GOLD COAST FUNGI. I.

By H. A. DADE

(With 4 Text-figures)

Thielavia setosa n.sp. (Gold Coast No. 555)

This fungus was found clambering over the aerial growths of mixed cultures of moulds growing on several samples of cacao "beans" which had been incubated in a damp atmosphere in Petri dishes. Here it was rendered conspicuous by its very profuse formation of characteristic ascocarps.

DESCRIPTION

When mouldy cacao beans are incubated in a humid atmosphere, a dense growth of Aspergilli forms on their surface, and above these there is commonly a luxuriant tangle of the aerial mycelium and sporophores of *Absidia* spp. In these conditions *Thielavia setosa* grows most vigorously and produces large and fully developed ascocarps. Its mycelium consists of fine hyphae which are strung, like spiders' silk, over and about the *Absidia* hyphae and among the heads of the Aspergilli. On these filaments the ascocarps are borne.

Where less aerial mould growth occurs, as in culture on agar, ascocarps appear on the surface of the medium; and when the competing fungi are predominant, they may be immersed in the medium.

The ascocarps are globose, brilliantly white and shining when young, later becoming brown, and black when mature, due to the dark brown or olivaceous colour of the cortical cells.

The most characteristic morphological feature of the ascocarp is the bristling array of appendages which project radially from all sides, like the spines of a sea urchin or the spicules of *Radiolaria*. These are fully developed in vigorous aerial growths, but may be few and poorly developed in other situations; in immersed ascocarps they are represented only by dark radiating hyphae of unspecialized form. When fully developed, the appendages are simple, rigid and acicular, with expanded and somewhat bulbous bases which fit into the mosaic of cortical cells. They pass through the same colour changes as the ascocarp itself.

In the development of the ascocarp, initials are formed from a short simple spiral of two or three turns, borne on a single hypha. The knot of investing hyphae which soon surrounds and conceals the spiral seems to be derived from one or two small laterals which spring from the stalk of the fertile hypha.

The asci are pear-shaped when young, becoming globose when full grown, with very thin transparent walls. Each contains eight ascospores.

The ascospores are hyaline at first and dark fuliginous or nearly black when mature. At each pole of the spore there is a pore in the exospore, through which the endospore protrudes slightly as a transparent papilla. The papillae cause the broadly fusiform spores to resemble lemons in form.

Germination is generally bipolar, though it may sometimes occur at one pore only. The germ-tubes are expanded where they emerge from the spore, but are not typically lop-sided like those of *Thielavia basicola* described by McCormick(1).

Aleuriospores, which are generally produced, are ovoid and transparent, either sessile on the sides of distributive hyphae, or borne terminally on short laterals. Occasionally they are terminal on long hyphae, the lower cells of which bear sessile spores. Their cell walls are somewhat thickened and refractive, and they may function as chlamydospores. Germination has not been observed.

Repeated search has been made for other spore forms, but none has been found.

CULTURAL CHARACTERISTICS

Attempts to isolate the fungus in pure culture were at first unsuccessful, the separation plates becoming covered by the contaminants before the desired growth appeared. Eventually clean cultivations were obtained by picking out ascocarps which had formed on the reverse side of filter paper used to keep the Petri dishes moist. These pure cultures showed very slow growth and produced only a few ascocarps, none of which contained spores.

One of these pure cultures became contaminated by a species of *Scopulariopsis*, and vigorous growth and an abundant crop of ascocarps ensued. This accident, indicating the Heald-Pool reaction, provided an early clue to successful cultivation, and several "commensals" were tried, *Aspergillus flavus* being the most satisfactory. It was then found that equally good results could be obtained by planting the fungus on slants of potato-dextrose agar previously occupied by young (three to six days old) cultures of *A. flavus* and resterilized by immersing the tubes in boiling water for about half an hour.

This recalled the work on *Thielavia basicola* by McCormick(1), who found that this species could be induced to fruit on media dosed with sterile filtrates containing fungic extracts. The same method succeeds with the present fungus, but the simpler method described above is equally effective, though McCormick found it useless for *T. basicola*. The age of the preliminary cultivation of *Aspergillus flavus* is important. It must be allowed to grow until sufficient of the substances

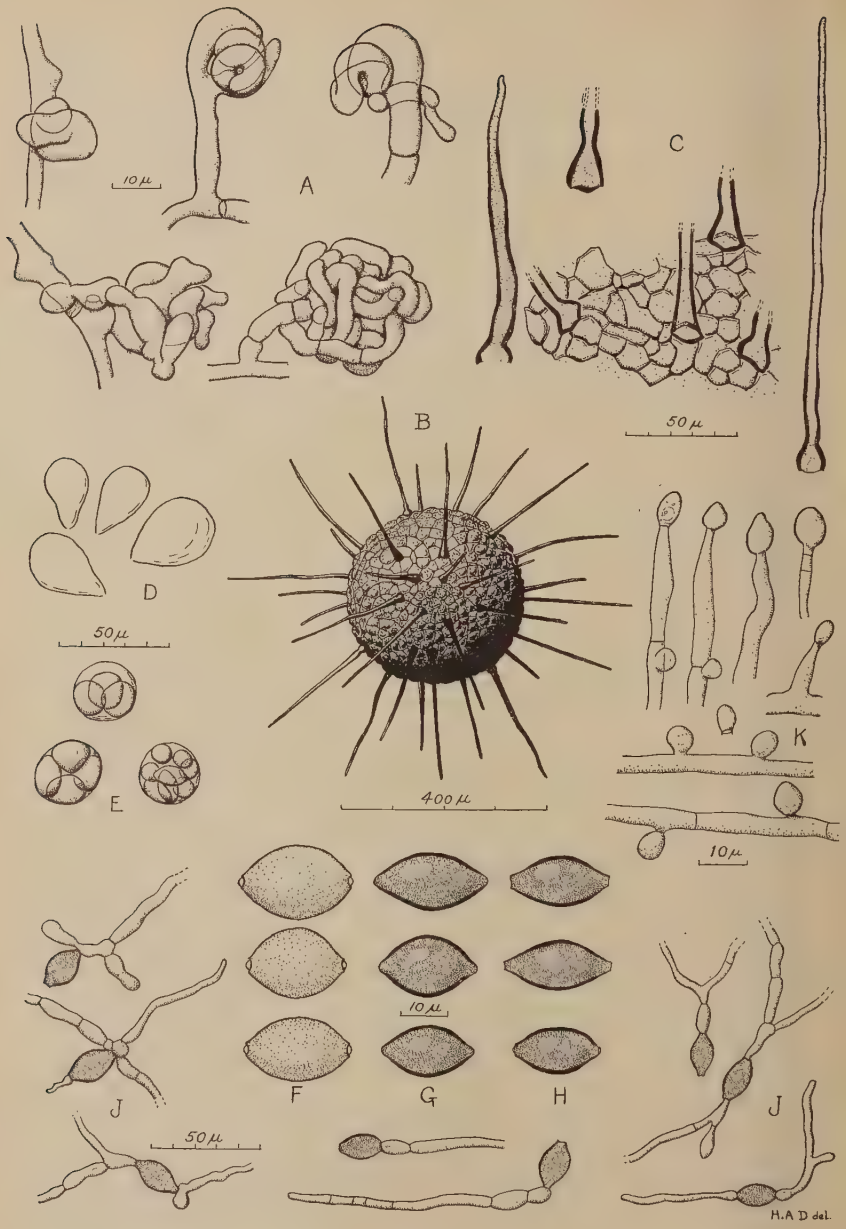

Fig. 1.

necessary for growth of *Thielavia setosa* has accumulated. Very young growths do not give good results. Presumably the extracts of *Aspergillus flavus* required by *Thielavia setosa* are not entirely destroyed by the short heating necessary to kill the former fungus.

In pure culture on virgin medium there is a slow growth of mycelium which forms a fine white or creamy felt. In the more vigorous of these vegetations a few fruit bodies may be formed, hidden within a thick, fluffy felted mass of hyphae, but they are not well developed and contain no spores, and attempts at subculture usually fail. Mass transfers from growths on sterilized *Aspergillus flavus* slants are somewhat more successful, probably because a sufficient quantity of *flavus* extract is carried over with the transfer, but again subcultures do not grow. These observations were made in the Gold Coast at laboratory temperatures ranging from 25 to 30° C. Cultures were sent to Mr R. H. Bunting in England, and in some he found that a good crop of ascocarps, with mature ascospores, was produced in the incubator at a temperature of 24° C. in about three weeks. This may indicate that the effect of temperature is similar to that in *Melanospora Zamiae* Corda, which in the Gold Coast will grow only with a commensal, or in the presence of extracts, like *Thielavia setosa*, while pure cultures sent to England grow freely to maturity with no stimulation by other fungi.

In mixed culture with living growths of the several other fungi which have been tried, *Thielavia setosa* always produces fertile ascocarps, but its reaction with its commensals varies. With *Aspergillus flavus* (typical strain from cacao) the two fungi, planted together at the centre of the plate, advance together, *Thielavia setosa* producing aerial hyphae, which clamber over the heads of the *Aspergillus*, and concentric rings of ascocarps. The fruiting of *A. flavus* is considerably repressed, but its vegetative growth keeps pace with that of its companion. The same proportionate growth occurs with the *Scopulariopsis* sp. previously mentioned. *Aspergillus Chevalieri* and *A. Tamarii* (cacao strains) are, however, completely suppressed by *Thielavia setosa*, and both fungi then cease to extend their centrifugal growth. On the other hand *Aspergillus niger* (cacao strain) can successfully compete with *Thielavia setosa*, the latter producing ascocarps only during the early growth of the dual planting, and sending out a few radial aerial hyphal threads for a short distance, after which it ceases to advance.

It is clear that while the substances produced by all these com-

Legend to Fig. 1.

Fig. 1. *Thielavia setosa* Dade. A, ascocarp initials, × 716 d.; B, fully developed mature ascocarp, × 78 d.; C, setae, and bases showing insertion on ascocarp wall, × 330 d.; D, young asci, × 330 d.; E, asci with developing ascospores, × 330 d.; F, maturing ascospores, from cocoa; G, mature ascospores, from cocoa; H, mature ascospores, from potato-dextrose agar, × 716 d.; J, germinating ascospores, × 330 d.; K, aleurio-spores, × 716 d.

mensals are favourable to *T. setosa*, there is competition, with varying results, for the nutrition provided by the substratum.

Similar reactions occur when the two fungi are planted at some distance from one another on the same plate. Normal pure culture growth occurs until the circumferences of the two vegetations meet. With a favourable commensal the hitherto feeble, sterile *T. setosa* advances rapidly in a fan centred on the point of contact, into the area occupied by the other fungus, and forms a profusion of perithecia. The companion fungus may continue to grow into the *T. setosa* area, but no ascocarps are formed on the sterile mycelium of the latter, where growth has ceased, and which is therefore no longer susceptible to stimulation.

Ascospores germinate quite well in hanging drops of normal potato dextrose and other fluids, but the percentage of germination is distinctly greater in a medium to which fungic extract has been added.

TAXONOMY

This fungus seems to be best included in the genus *Thielavia*, from the other species of which it differs in the presence of well-developed appendages on the ascocarps and in the very large ascospores. The latter are, however, typical of the genus in form. Only one other species—*T. Sepedonium* Emmons (2)—has aleuriospores. In its dependence for development, at least at high temperatures, on the presence of extracts of other fungi, *T. setosa* strongly resembles *T. basicola*.

It is of interest to note that similar nutritional phenomena occur in the genus *Melanospora*. Moreover, the ascospores and terminal chlamydospores of *M. Zamiae* (3) strongly resemble the ascospores and aleuriospores of *Thielavia setosa*. There is also a remarkable resemblance between the young ascocarps of *Melanospora Zamiae* and *Thielavia setosa*. These two genera are undoubtedly closely related.

DIAGNOSIS

Thielavia setosa n.sp.

Ascomata typice aeria vel superficialia nonnunquam immersa; globosa, sine ostiolo, nigra, membranacea, 150–300μ, plerumque 250–300μ diam., cum appendicibus multis radiantibus. Appendices rigidae, aciculares, 150–300μ longae, 8–19μ crassae, basi dilatae 12–16μ crassae.

Asci globosi, subtiles, 8 sporas continent.

Ascosporae fuligineae vel subnigrae, citriformes, 20–25μ longae, 13–15μ latae, papillis polaribus hyalinis.

Aleuriosporae ellipticae vel ovatae, hyalinae, ad 8μ longae, 7μ latae, sessiles vel in hyphis brevibus lateralibus terminales.

Aliae sporae ignotae.

Saprophila in seminibus *Theobromae Cacao* L. in Gold Coast, Africae Occidentalis.

Syncephalis nana n.sp. (Gold Coast No. 570)

This fungus was found as a parasite on a strain of *Absidia Regneri* (Lucet & Cost.) Lendner, which is a mould of inadequately dried cacao beans in the Gold Coast. The samples of cacao beans in which the parasite appeared were from Kpeve, in the British Mandated Territory of Togoland.

In the course of work on cacao moulds, samples of the split beans were incubated on moist filter paper in Petri dishes, and a luxuriant aerial growth of the *Absidia* was obtained. Examination with a binocular magnifier disclosed the presence, among the *Absidia* mycelium, of a small organism with minute yellow heads. This was studied in the original growth and in subsequent cultures, free from other fungi, on artificial media.

The aerial mycelium of the host becomes invested with a fine web of delicate hyphae which form a network, with swollen anastomoses, typical of *Syncephalis*. Fruiting in the *Absidia* is inhibited to some extent, and the stolons and sporangiophores of the host become packed with fine intracellular hyphae.

The fertile hyphae of the parasite form rather short sporangiophores, thick at the base and tapering to the insertion of the inflated apex. A well-developed foot with rather short thick rhizoids firmly clasps the host hypha. The head, or sporangium, is pear-shaped, somewhat flattened on top, and tapering below into the stalk. On the rather flattened apex the sporangium bears between thirty and forty "partial sporangia" (Gäumann's term (4)). These are carried on short sterigmata, which appear as warty protuberances on heads from which the spores have fallen.

The partial sporangia are narrowly ampulliform at first—resembling the phialides of *Aspergillus*—and subsequently take on a form determined by the contained spores. Each contains two spores, shaped like short cucumbers, and set together in a line which follows the curvature of the spores. The concave side of the pair faces inwards, and the complete set of partial sporangia therefore assumes a barrel shape. The colour of the mature spores is that of clear honey. The pairs of spores separate when they are shed. The cluster of partial sporangia frequently falls from the head in a mass, with some tendency to remain adhering in a barrel-shaped bundle for a time.

Zygospores have not been found.

The fungus is easy to maintain in culture with its host, and appears after the *Absidia* has made a good growth. It is a highly specialized parasite, for it does not destroy nor seriously depress its host. Prepara-

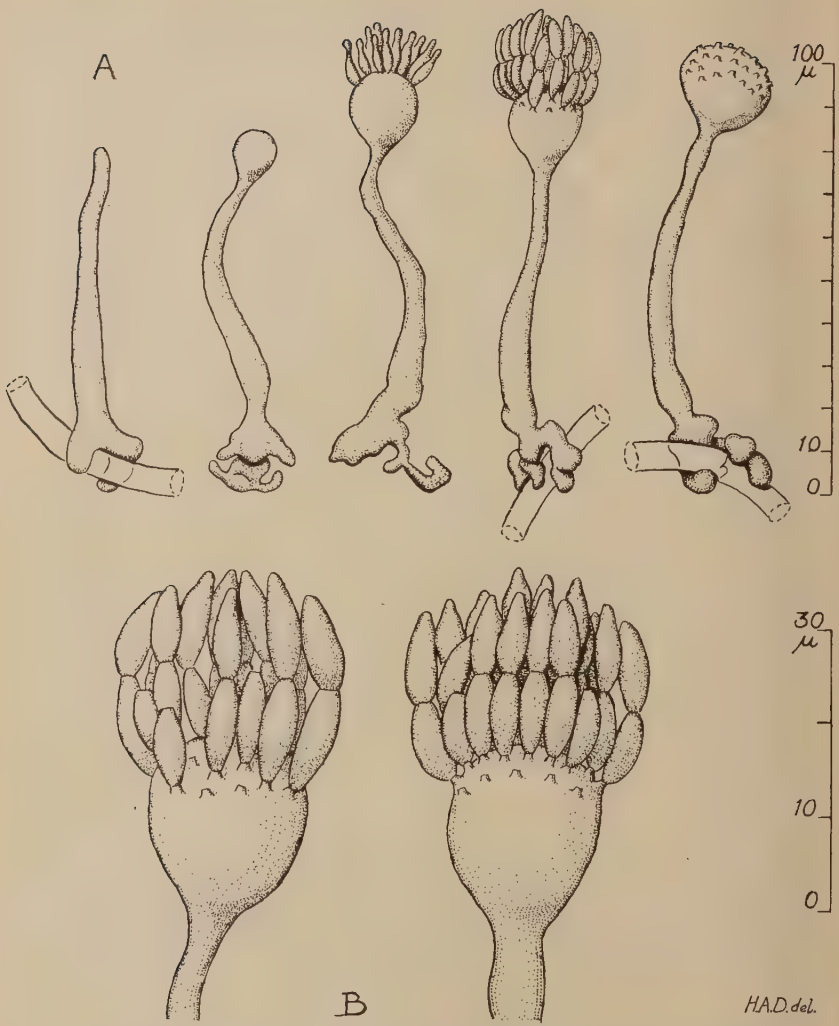

Fig. 2. *Syncephalis nana* Dade. A, complete developing and mature sporangiophores, × 660 d.; B, inflated apices of sporangiophores, bearing clusters of "partial sporangia" and spores, × 1432 d.

tions in Amann's medium, with which cotton blue is incorporated, successfully demonstrate the parasite, for the mycelium, especially the fertile hyphae, takes up the cotton blue more readily than does the *Absidia*.

TAXONOMY

There is no species resembling this fungus among those described by Fischer (5). The nearest in Fischer's list is *Syncephalis sphaerica*, with undivided "basidialconidien" and rod-shaped spores, but these spores are mostly in five-jointed straight chains. *S. fusiger*, with chains of two spores each, has compound "basidialconidien".

Thaxter (6) describes a species, *S. tenuis*, with "fertile hyphae septate at base, very elongate, tapering to a slender extremity which expands abruptly to form the fertile head, the latter somewhat flattened and bearing from six to many sporangial filaments arising from all parts of the upper surface or arranged in a more or less definite circle, each producing two spores. Spores subcylindrical to asymmetrically oval, truncate or bluntly rounded, cylindrical forms 20–25 × 7μ, oval forms 25–27 × 7μ at base to 4–5μ at apex. Sporiferous head without spores 10–20μ diam."

This is much closer to the present fungus, but the latter is smaller and has by no means a "very elongate" stalk. These differences are considered to be sufficient to warrant the recognition of a new species, for which the name *Syncephalis nana* is proposed.

DIAGNOSIS

Syncephalis nana n.sp.

Mycelium cum nodis tumidis reticulatum, typicum generis.

Hyphae fertiles cum capitibus inflatis pedibusque bene formosis, prope basin 8μ diam., apice 2–3μ diam., hyalinae, in maturitate flavidae. Pedes simpliciter ramosi, qui hyphas hospitis complexi sunt. Vesiculae piriformes, 15–20μ crass., in apicibus planulis 30–40 sporangia ferentes.

Sporangia 16–22μ longa, subcylindrica curvulata, in sterigmatibus brevibus, 2 sporas continent.

Sporae a sporangiis secedentes et saepe in fasciculum doliiformem cohaerentes, fusiformes curvulatae vel cucumeriformes, melleae, 10–12μ long., 4μ crass.

Zygosporae ignotae.

Parasitica in *Absidia Regneri* (Lucet & Cost.) Lendner in Gold Coast, Africae Occidentalis.

MUCOR INAEQUISPORUS N.SP. (Gold Coast No. 637)

At Aburi, in the Gold Coast, ripe fallen fruits of the hog plum, *Spondias monbin* L., soon become covered with a velvety orange-yellow mould. This was found to be a *Mucor* with the following characteristics.

The sporangiophores are typically of *Monomucor* form—i.e. simple unbranched aerial hyphae with terminal sporangia. Rarely, a small lateral branch and sporangium are produced. On fruit of hog plum the fertile hyphae are from 9·5 to 0·75 cm. in length; on potato-dex-

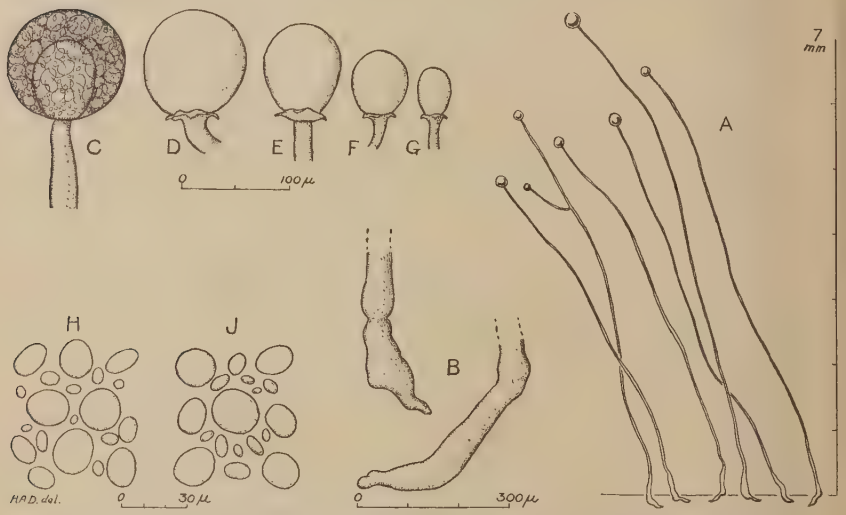

Fig. 3. *Mucor inaequisporus* Dade. A, habit, × 10 d.; B, bases of sporangiophores, × 78 d.; C, a mature sporangium, × 165 d.; D–G, columellae, × 165 d.; H, J, spores from vegetations on hog-plum fruit and potato-dextrose agar respectively, × 330 d.

trose agar, when growth is unrestricted on plates covered with bell-jars, they attain a height of 4 cm.; their diameter is 35–49μ, and they have broad short bases 40–70μ diam.; they have orange-coloured contents. The sporangia are spherical, averaging 100–150μ diam. on the fruit, maximum 200μ; on potato-dextrose agar the average diameter is 100μ, maximum 150μ; they are golden brown when young, dark brown when mature, by reflected light; the membrane is diffluent, and has no investment of crystals. The columellae are pyriform, subspherical in large individuals, ranging from 40 × 30 to 100 × 90μ, average about 80 × 60μ, with rather deep brownish orange contents.

The spores are exceedingly variable in size (the outstanding characteristic of this species), $3{\cdot}5 \times 2{\cdot}5$ to $25 \times 20\mu$, the smallest being elliptic, sometimes reniform, the largest nearly round, the intermediate sizes broadly elliptic; spores of all sizes come from each individual sporangium; they are pale yellowish and are not mingled with oil droplets.

The gross colour of vegetations is similar in culture on potato-dextrose and corn-meal agar and on hog-plum fruits, and strongly resembles the colour of the latter: Ridgway IV.19.—apricot to IV.19.*b* light cadmium-yellow. The fungus grows more freely on potato-dextrose than on corn-meal agar.

The long aerial sporangiophores are strongly heliotropic.

TAXONOMY

This species is not closely comparable with any other previously described. In spite of the occasional side branches, it is as typically a *Monomucor* as is *Mucor Mucedo*, which also produces lateral branches occasionally. In other features it also resembles *M. Mucedo*, to which it is evidently more nearly related than to other species; but it differs from *M. Mucedo* markedly in the colour of the sporangia and sporangiophores, in the form of the columella, the absence of crystals on the membrane, the more extreme variation in spore size, and the different shape of the spores.

These differences are considered to justify the proposal of a new species, *M. inaequisporus*.

DIAGNOSIS

Mucor inaequisporus n.sp.

Hyphae fertiles erectae sed heliotropicae, typice non ramosae, aliquando cum ramulis singulis lateralibus, hyalinae sed materiam aurantiacam continentes; in fructibus hospitis 0·5–0·75 cm., in agaro ad 4 cm. long., 35–49μ crass., basibus brevibus latisque 40–70μ crass.

Sporangia globosa, in juventate mellea, in maturitate badia, cum membrana diffluenti, crystallis carentia; in fructibus plerumque 100–150μ, ad 200μ diam., in agaro plerumque 100μ, ad 150μ diam.

Columellae piriformes vel si magnae subglobosae, hyalinae sed materiam aurantiacam continentes; $30–40 \times 90–100\mu$, plerumque $60 \times 80\mu$.

Sporae in sporangiis singulis magnitudine dissimiles, plerumque ellipsoideae vel magnae subglobosae vel parvae reniformes, subflavidae, guttulis olei carentes, $3{\cdot}5 \times 2{\cdot}5–25 \times 20\mu$, plerumque $20 \times 15\mu$.

Cultus in agaro armeniacus.

In fructibus maturis abstrictisque *Spondiadis monbin* L. in Gold Coast, Africae Occidentalis.

Absidia cristata n.sp. (Gold Coast No. 554)

This fungus was isolated from cassava stems on which it was saprophytic following anthracnose, and from fermented cacao on which it is an uncommon mould. In culture it is characterized by two distinct types of growth—it first forms a low sward of short, rather thickly branched sporangiophores, and later long cobwebby sporangiophores with similar racemose branching, but with the laterals at much longer intervals. It was at first thought that two fungi were involved, but observation in culture showed that the two forms belong to the same species.

The fertile hyphae are hyaline, up to 20μ thick, the lateral branches narrower, up to 8–9μ thick. The sporangia are nearly spherical, but with the infundibuliform apophysis typical of *Absidia*, and dark brown to black in appearance. The columellae are spherical to oval, of diverse form: in terminal sporangia they are large, globose, up to 40μ diam., smooth on the summit; in smaller lateral sporangia they are oval, with a distinctly formed papilla on the summit; while in the smallest laterals they are narrowly oval, nearly twice as long as broad, with one or a few apical processes which may be simple papillae but are more often highly developed outgrowths of more or less complex form; all columellae are fuliginous, the colour extending into their infundibuliform bases. The spores are irregularly rounded, with a tendency to polygonal outline, 4–7μ diam. and smoky brown; there is a suggestion of punctate markings. The zygospores, chlamydospores and gemmae have not been seen. In culture abnormal growths occur, taking the form of giant hyphae and of large tuberculate developments of sporangial heads, all with dense granular contents.

Of the species of *Absidia* previously described, *A. Lichtheimii* (Lucet & Cost.) Lendner(7) seems to be nearest to the present fungus. An authentic culture of *A. Lichtheimii* was provided by the Imperial Mycological Institute for comparison. This produces smaller colourless spores 2–3μ diam., the columellae are more ovoid in form, and the apical processes are only very slightly developed. It is difficult to find any close resemblance between the two fungi, and it therefore seems proper to describe the Gold Coast fungus as a new species, with the name *A. cristata*.

Legend to Fig. 4.

Fig. 4. *Absidia cristata* Dade. (A–D, *A. cristata*; F–G, *A. Lichtheimii* for comparison.) A, habit, compact type, × 156 d.; B, abortions produced in culture, × 156 d.; C, large terminal sporangia, × 660 d.; D, a series of columellae, the largest from a terminal sporangium, the others from smaller lateral sporangia, × 660 d.; E, spores, × 660 d.; F, a series of columellae of *A. Lichtheimii*, × 660 d., for comparison with D; G, spores of *A. Lichtheimii*, × 660 d., for comparison with E.

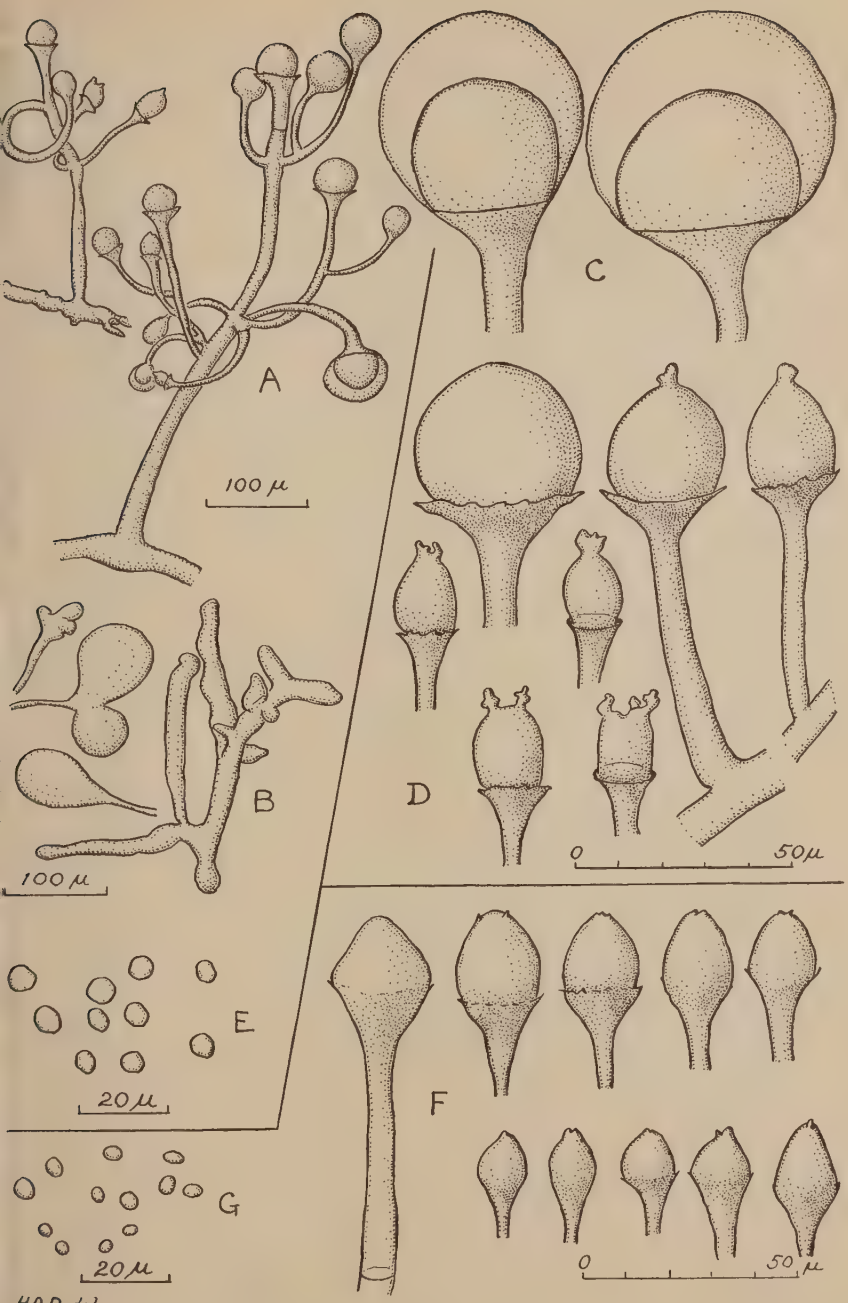

Fig. 4.

DIAGNOSIS

Absidia cristata n.sp.

Hyphae fertiles hyalinae racemosae biformes: 1. breves dense racemosae; 2. elongatae; sporangia magna terminales et minores in ramis lateralibus gerentes. Primae stirpes ad 20μ, rami laterales ad 8–9μ crass.

Sporangia pyriformia vel subglobosa, ad 60μ diam., apophyse infundibuliformi, in maturitate badia vel subnigra.

Columellae subglobosae vel ovales; in sporangiis terminalibus magnae, ad 40μ crass., cum apicibus levibus; in sporangiis lateralibus minoribus ovales, semper cum apicibus papillatis; in sporangiis lateralibus minimis subanguste ovales, singulos vel paucos, simplices vel saepius magnas multiformes, apicales processus gerentes; fuliginosae.

Sporae incondite globosae vel ovales, subpolygoniae, aliquando paululum punctulatae, fumosae-fuliginosae, 4–7μ diam.

Zygosporae ignotae.

Saprophila in stirpibus *Manihotis utilissimae* Pohl. et in seminibus *Theobromae Cacao* L., in Gold Coast, Africae Occidentalis.

The author is indebted to Messrs S. F. Ashby, E. W. Mason and R. H. Bunting for assistance in consulting literature not available in Africa, and for their opinions on diagnoses.

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A PYTHIUM WILT OF PRIMULA CAUSED BY *PYTHIUM SPINOSUM*, SAWADA

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(With 7 Text-figures)

Introduction

In October 1934 a serious epidemic disease occurred in the experimental plants of *Primula sinensis* growing at the John Innes Horticultural Institution. The seedlings wilted in the seed pans, causing considerable mortality among the many hybrid varieties grown for experimental purposes. A preliminary examination of the seedlings was made by Dr G. H. Pethybridge, who came to the conclusion that the epidemic was due to the parasitic fungus, apparently a member of the Pythiaciae, which he found growing in the roots. Some of the material was sent to the senior author with a request that he would make a more detailed investigation of the fungus and determine its identity if possible.

Meanwhile, investigations carried out by Miss D. M. Cayley at the John Innes Horticultural Institution showed that, although finally the seedlings became infected by a fungus, the immediate cause of the disease was the soil in which the *Primula* plants were growing. This soil was sterilized for forty minutes at 100° C. and then left for about six weeks in a soil bin outside before it was used for potting the seedlings. Miss Cayley's work indicated that heating the soil broke down the humus of the compost, rendering the soil too dense and thus preventing normal growth of plants in it. "The main cause of the apparent epidemic of wilt is that the roots of the plants are not keeping pace with the top growth, and as they grow larger the excessive evaporation makes them wilt and collapse and thus become an easy prey to secondary organisms".* Miss Cayley tried the effect of repotting seedlings which were attacked, in smaller pots in a much more porous soil, with the result that, within a few weeks, the plants recovered. Similar results were obtained with infected plants which had been sent to the authors and which were treated in the same way in the experimental greenhouse at Cardiff. In such plants the spread of the fungus is arrested.

It was felt, however, that it was still desirable to determine the nature of the fungus in the roots, since a preliminary study had failed to identify it.

* In a letter to the senior author.

Methods

The material received consisted of fresh seed and infected seedlings, some of them still in their original pots of infected soil. Some of the seedlings which were planted in infected soil have remained infected though they have not died. Others, which were repotted into the sterile soil used at Cardiff, grew healthily, and all signs of the fungus disappeared. Seed sown, both in the sterile soil and also on agar plates, never contracted the fungus, neither was any fungus isolated from the agar itself, although it was found that the infecting fungus would thrive on the medium. From this it was concluded that the fungus was present in the soil and did not come from the seed of *Primula sinensis*.

The fungus was isolated from infected roots by washing them in mercuric chloride and then teasing out the inner parts of the roots into sterile distilled water contained in small glass jars. After a few days growth appeared on pieces of hemp seed which had been introduced into the jars and the fungus passed through its entire life history at laboratory temperature under these conditions. Several separate isolations were made and the same organism was always isolated.

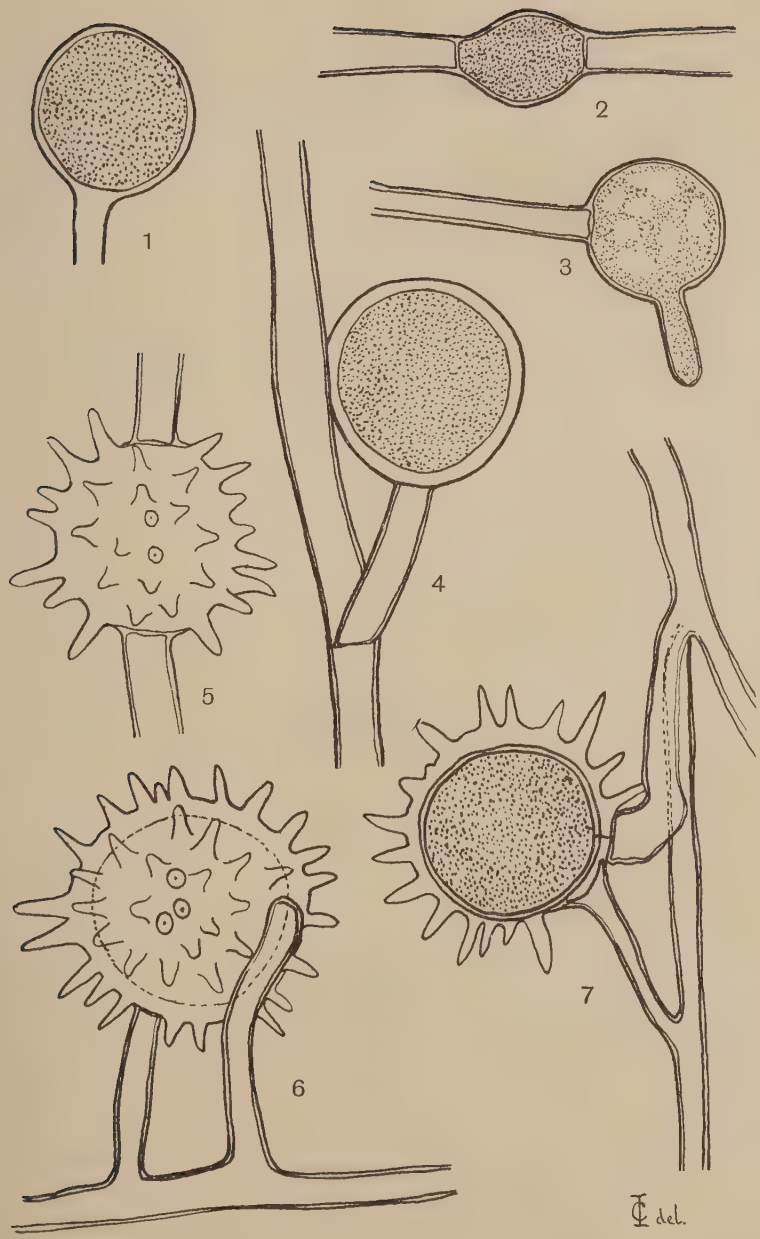
Life history of the fungus

The organism develops a fine mycelium forming a rather tufted growth on the hemp seed. The delicate hyphae measure 2–5μ diam. and, when young, are devoid of transverse septa. Septa appear in older hyphae, and in old mycelia regular septation is present. After a short time in culture the fungus begins to reproduce asexually by means of conidia. No zoosporangia are formed and zoospores are never differentiated. The conidia form abundantly in culture and usually develop terminally (Figs. 1—4), though intercalary conidia

Legend to Figs. 1–7.

The drawings were made with a camera lucida at table level using a Zeiss 4 mm. objective and compensating ocular × 20; tube length of 160 mm. giving a magnification of 1580. Reproduction is without reduction.

Fig. 1. A single apical conidium terminating a main hypha.
Fig. 2. An intercalary conidium at a young stage in development.
Fig. 3. A terminal conidium germinating by means of a single germ-tube while still attached to the parent hypha.
Fig. 4. A conidium developing on a lateral branch hypha, which has been cut off by a septum: the type of development on an older mycelium.
Fig. 5. An intercalary oogonium at a young stage in development; no antheridium was present at this stage.
Fig. 6. A mature oogonium and antheridium both arising from the same main hypha, but the antheridium not springing from the oogonium stalk. The dotted line indicates the position of the oosphere.
Fig. 7. A mature oogonium and antheridium after discharge of the contents of the latter into the oogonium. The drawing shows the appearance in median section with the oospore delimited. The antheridial stalk springs from a triple branch of the main hypha.



Figs. 1–7.

appear rarely. The wall of the conidium is usually smooth, though sometimes short spines may be developed. The conidia are roughly spherical and measure 16·5–34·3μ in diameter (average 26·4μ).* They germinate by means of a single germ-tube (Fig. 3) and never by the production of zoospores.

In older cultures sexual reproduction occurs. The antheridia are stalked, androgynous, and rarely arise from the oogonial stalk. The oogonia, which are usually formed terminally, are spherical, measuring 16·0–28·4μ (average 19·0μ), and densely covered with spines from 3·5–8μ long (Figs. 5–7). Each oogonium, after fertilization, gives rise to a single oospore which usually completely fills the oogonium. It is spherical and measures 13·0–20·3μ diam. (average 15·4μ). Its wall is smooth. Germination of the oospore has not been observed.

Discussion

The fungus is a member of the genus *Pythium*, most closely agreeing with *P. spinosum*, a culture of which was obtained from the Centraalbureau voor Schimmelcultures: the following table shows the comparative measurements:

	Pythium spinosum Sawada	Fungus isolated from seedlings
Hyphae	2–5μ diam.	2–5μ diam.
Conidia	14–33μ (av. 22·4μ) diam.	16·5–34·3μ (av. 26·4μ) diam.
Oogonia	17–24μ (av. 19·7μ) diam.	16·0–28·4μ (av. 19·0μ) diam.
Spines	5–8μ long	3·5–8μ long
Oospores	12–21μ (av. 16·6μ) diam.	13·0–20·3μ (av. 15·4μ) diam.

These comparisons show that the fungus which attacked the seedlings was *Pythium spinosum* Sawada. This species was first described by Sawada & Chen (3) as a parasite of *Antirrhinum* seedlings in Formosa. They showed by inoculation experiments that the fungus could infect young plants of a number of other common species, and its ability to attack the seedlings of *Primula sinensis* is in no way remarkable. The important question, however, is whether this species has been introduced into this country from Japan. We are informed that records from the John Innes Horticultural Institution show that no plants had been introduced from Japan during a period which would make infection probable. It seems likely therefore that the species occurs wild in this country, but that it is only by chance that its presence has become recognized. The genus *Pythium* has as yet been only superficially investigated in Great Britain, and experiments carried out in the Department of Botany at Cardiff indicate that the number of common species inhabiting the soil is far greater than is generally recognized, and that a number of species apparently not so far recorded for this country have been found.

* Each average is based upon twenty-five measurements.

The occurrence of this species as an epidemic parasite of *Primula* seedlings, however, seemed sufficient to justify special notice being taken of the fungus.

Specimens of *Pythium spinosum* Sawada, both from the Baarn culture and also from the isolation on Primulas, have been preserved in the senior author's herbarium.

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NOTES ON ENTOMOGENOUS FUNGI

By T. PETCH

101. *Entomophthora Planchoniana* Cornu

Cornu, in *Bull. Soc. Bot. France*, xx (1873), 189, 190, gave an account of an *Entomophthora*, found on an aphid on elder at Montpellier, for which he proposed the name *E. Planchoniana*, because apparently the same fungus had been observed previously by Planchon on an aphis on a vetch. No formal description was given, and the following details give all the relevant information.

The affected insects were brick-red, instead of the normal black, and their abdomens were enormously swollen. The sporangia were globose, apiculate, filled with a refringent plasma, and containing in the centre a spore of the shape of a "toupie d'Allemagne". The fungus resembled *Empusa Muscae*, but could not be referred to that species. In a moist atmosphere the sporangia produced secondary sporangia, similar to the primary. Under special conditions of humidity the wall of the sporangium remained adherent to the wall of the spore, and the shape of the whole was then ovoid. In a subsequent paper by Brongniart & Cornu (*Ass. Franç. Avanc. Sci.* Paris, 1878), which deals with an attack of an *Entomophthora* on syrphids, there is a footnote, stating that according to the degree of humidity *E. Planchoniana* produces ovoid-oblong spores without sporangia, or sporangia soldered to the spore.

By "sporangia" Cornu meant what are now known as conidia. The true conidium of an *Entomophthora* is formed within a mother cell, the wall of which is normally in close apposition to that of the conidium, so that the whole appears to be a single spore. But in some species, with excess of moisture, the wall of the mother cell separates from the conidium proper, so that the latter floats free in a large spherical cell. As regards the shape, "toupie d'Allemagne", according to the dictionaries, means a humming top. But in the second note referred to above the same term is applied to the conidium of an *Entomophthora* which was undoubtedly *Empusa Muscae*. It is clear, therefore, that *Entomophthora Planchoniana* had conidia which resembled those of *Empusa Muscae*, and under suitable conditions of humidity the outer wall separated from the true conidium, leaving the latter floating in a large spherical cell.

Thaxter, in *Entomophthoreae of the United States* (1888), applied Cornu's name to an American species "with much hesitation". He pointed out the discrepancies between Cornu's two descriptions of the spore, but apparently did not realize that Cornu's "toupie d'Alle-

magne" was the same shape as the spore of *Empusa Muscae*. Thaxter's American species had globose spores, and he stated that he had never seen a separation of the outer wall in spores of that type.

In September 1933, I found a single specimen of an *Empusa* on a detached aphid in Deepdale, Barnard Castle, which resembled *E. Muscae*, and was recorded under that name. Subsequently, I received specimens of the same fungus on an aphid on raspberry from Dr H. S. Fawcett, collected at Puyallup, Washington, U.S.A., June 1934; and in May 1935 I found it in quantity on an aphis on greengage in my garden at North Wootton.

The insect is fastened to the leaf by short rhizoids from the under side of the body, which are not visible until it is forcibly detached. The conidiophores cover the insect either in a continuous even stratum or in irregular confluent masses. The insect is red, and the layer of conidiophores is pale reddish. The conidiophores are simple and there are no cystidia. The conidia are ejected with a drop of plasma, as in *E. Muscae*, are bell-shaped or globose with a truncate base, and are furnished with an apical point; the sides are turned outwards slightly towards the base, which is slightly convex; they measure 17–23 × 12–20μ. In a spore print in a damp chamber, the outer wall frequently separates from the conidium proper, so that the latter floats free in a spherical cell about 30μ in diameter. The secondary conidia are the same shape as the primary, with or without an apical point, but smaller, 15–20 × 12–14μ, and are produced by direct budding from the primary conidia. The spore is shaped like an old-fashioned German spiked helmet.

This species appears to fit Cornu's description better than that for which the name was adopted by Thaxter. It resembles *E. Muscae*, but has smaller conidia, and the insect is attached to the plant by rhizoids. In spite of the latter feature, however, it must be referred to *Empusa*, not *Entomophthora*, as the conidiophores are simple. I propose to adopt Cornu's name for this species, viz. *Empusa Planchoniana* (Cornu) Petch non Thaxter, while the species for which the name was employed by Thaxter may be known as *E. Thaxteriana*.

In *Ann. Mag. Nat. Hist.* Ser. 5, XVIII (1886), 4, pl. 3, figs. 1–13, Phillips recorded *Entomophthora ferruginea* on *Aphis rumicis*. The fungus had been compared with *Entomophthora Aphidis*, and found to be different. The details given are not sufficient for an accurate determination, and the drawings are equally inadequate, but some of the latter show narrow bell-shaped conidia, sometimes with an apical point, and could be matched in a squash preparation of *Empusa Planchoniana*. *Entomophthora ferruginea* would appear to agree better with *Empusa Planchoniana* than with *Entomophthora Aphidis*, to which Thaxter referred it. Unfortunately, no specimen has been found in the Phillips collection in Herb. British Museum.

The synonymy of the two species considered above is:

Empusa Planchoniana (Cornu) Petch; *Entomophthora Planchoniana* Cornu in *Bull. Soc. Bot. France*, xx (1873), 189; *Entomophthora ferruginea* Phillips in *Ann. Mag. Nat. Hist.* ser. 5, xviii (1886), 4; non *Empusa Planchoniana* Thaxt., *Entomophthoreae of the United States* (1888), p. 165.

Empusa Thaxteriana Petch, nom.nov.; *Empusa Planchoniana* Thaxter, *Entomophthoreae of the United States* (1888), p. 165.

102. *Entomophthora Pyralidarum* Petch, n.sp.

In December 1923, large numbers of "grass moths" (Pyralidae), which had been attacked by an *Entomophthora*, were found in the Arboretum, Royal Botanic Gardens, Peradeniya, Ceylon, attached to the trunks of trees. A similar occurrence was observed at Vavuniya, in the low-country, in the same month; and in the following month examples were found at Kandy. Specimens were submitted to Prof. Thaxter, who stated that he was not acquainted with the fungus.

The moths were attached to the bark by numerous rhizoids, which were simple, sometimes expanding towards the extremity, and arose chiefly from the abdomen. The conidiophores are branched, and form a loose stroma, principally over the abdomen, becoming aggregated in small tufts. The conidia are pyriform, or oval with a conical or obtuse papilla, 16–28 × 12–16μ, or globose, 18–28μ diameter, with a conical papilla, 4–8μ high, not apiculate. Oblong, thick-walled chlamydospores, with a large central gutta, 22–28 × 13–14μ, occur terminally on the hyphae. Internally, zygospores (?), spherical, smooth, hyaline, 14–24μ diameter, were found. The contents of the conidia are uniformly granular.

Entomophthora pyralidarum Petch, n.sp.—Hyphis rhizoideis simplicibus, interdum apicem versus incrassatis, praecipue ex abdomine oriundis; conidiophoris ramosis praecipue abdomine insessis, fasciculatis; conidiis pyriformibus vel ovalibus, conice vel obtuse papillatis, 16–28 × 12–16μ, vel globosis, 18–28μ diam., papilla conica, 4–8μ alt., instructis; chlamydosporis oblongis, episporio crasso, terminalibus, 22–28 × 13–14μ, gutta magna centrali; zygosporis (?) internis, sphaericis, levibus, hyalinis, 14–24μ diam. In Pyralidis, Ceylon.

103. *Hypocrella cornea* Petch, n.sp.

Five gatherings of this species, on an Aleyrodid on *Rubus*, were included in a collection of entomogenous fungi from Kwangsi Province, China. In general appearance it resembles *Hypocrella Mollii*, but differs from that species in texture. It is white or yellowish, but becomes amber-coloured when rubbed, having a cortical zone which is hard and horny when dry. There are indications that it may be viscid when fresh. The ostiola are dark amber, not brown as in *H. Mollii*.

Hypocrella cornea Petch, n.sp.—Stromatibus usque 2·5 mm. diam., orbicularibus, discoideis vel depresso-pulvinatis, aequalibus vel leniter tuberculatis, albis vel flavescentibus, pruinosis, cortice sicco duro corneo, medio albis, laxis; peritheciis immersis, praecipue ad marginem, late ampullaceis, 0·3 mm. alt., 0·2–0·25 mm. diam., pariete hyalino vel flavo; ascis cylindraceis vel fusoideis, 130–200 × 8–10μ; articulis sporarum cylindraceis, dein ovalibus, truncatis, 7–9 × 1·5–2·5μ. On an Aleyrodid on *Rubus*, Kwangsi Province, China.

104. *Cordyceps formicivora* Schroet.

In *Krypt.-Flora von Schlesien*, III, 2 (1894), 276, Schroeter described *Torrubia formicivora*, which had occurred on *Formica ligniperda* in Silesia. The stroma consisted of a very short stalk, 1–2 mm. long, 0·5 mm. diameter, sometimes almost lacking, and a globose or hemispherical head, up to 2 mm. diameter, blackish, brownish internally. The perithecia were crowded, oval, up to 0·3 mm. diameter, semi-immersed, with a conical ostiolum. The asci were cylindric, generally attenuated below, 150–170 × 6–7μ, eight-spored, the ascospores being linear, 100–110μ long, 2·5–3μ broad, at first continuous, then septate, hyaline or pale yellow. The conidial clava was awl-like, simple, or divided above with diverging branches, up to 3 mm. high, 0·5 mm. diameter, blackish brown, generally pale violet-brown at the apex. Schroeter's fungus was clearly not *Cordyceps myrmecophila* Ces., and his description did not suggest any previously known species.

In *Proc. Amer. Phil. Soc.* LXXIV (1934), 263, Prof. E. B. Mains recorded *C. formicivora* Schroet., noting: "This curious species has been reported by Povah. He obtained one specimen on an ant at Rock River, Michigan, in 1927. This is apparently the first report for North America. Povah, however, states that Prof. Thaxter had collected it on several occasions at York, Maine."

I have examined specimens collected by Thaxter at Kittery Point, Maine, August 1892 (Farlow Herb. 6149), and at York, Maine (Farlow Herb. 6154), all on ants.

In No. 6149, the ants are fastened to the substratum by brown mycelium, with rather loose brown mycelium along the sutures, legs, etc. One has two short brown clavae arising from behind the head, each bearing a black, verrucose, lateral, perithecial plate. The other has three adjacent perithecial plates behind the head, but the clavae are so short that the plates appear sessile.

In No. 6154, one specimen has two shortly stalked perithecial plates behind the head. The other bears two clavae, one from behind the head, 3 mm. high, with two lateral perithecial plates at the base, and conidia on the upper linear part of the clava, and the other, conidial only, arising from a leg. The conidial clavae bear *Hirsutella* conidiophores.

Thaxter assigned these specimens to *Cordyceps unilateralis*, but subsequently crossed out that name. But they are, I think, that species, though smaller than is usual in the tropics. There is another North American specimen of *Ophiocordyceps unilateralis* in the Thaxter collection, Cullowhee, N.C., 15 June 1896 (Farlow Herb. 6161).

Rereading Schroeter's description in the light of these specimens, it seems clear that he was dealing with a small example of *Ophiocordyceps unilateralis*. The American specimens have been correctly assigned to Schroeter's species, but his name must be regarded as a synonym. His record appears to be the only reported occurrence of this species in Europe.

105. *Cordyceps superficialis* (Peck) Sacc.

In the 28*th Report N.Y. State Museum*, p. 70, Peck described *Torrubia superficialis*, which he stated was intermediate between *Cordyceps acicularis* and *C. Ravenelii*. As these two latter are the same species, an *Ophiocordyceps*, Peck's name was reduced to a synonym in *Trans. Brit. Mycol. Soc.* xviii (1933), 60.

Peck's type has now been examined by Prof. E. B. Mains, who has published the following redescription of it in *Proc. Amer. Phil. Soc.* lxxiv (1934), 268.

"Stromata 'vinaceous-buff', 2·5 and 3·5 cm. long, 1·5–2 mm. in diameter, the stipes 10 and 15 mm. long, fertile portions 15 mm., one with sterile acuminate apex, 5 mm. long, the other with apex apparently broken off; perithecia superficial, free, somewhat crowded, 'army-brown', ovoid, 456–660 × 450–564μ, some with apex collapsed or broken off; asci cylindric, 170–230 × 7–9μ; ascospores hyaline, filiform, probably about equalling the asci in length, breaking into segments 14–30 × 1·5–2μ."

In the Farlow Herbarium there is a specimen, No. 6130, collected by Thaxter, "on *Melolonthae* (?) in mossy woods, West Haven, Conn., 26 July 1889", which is apparently referable to Peck's species. The clavae are up to 2·5 cm. high, with a flexuose, longitudinally grooved, smooth, terete, brown stalk, 0·5 mm. diameter. The fertile portion of the clava is about 1 cm. long, 1 mm. diameter, cylindric, dark brown, almost black. Sometimes the stem is continued above as a sterile yellow apex. The perithecia are superficial, crowded, ovate, 0·36–0·4 mm. high, 0·3–0·36 mm. diameter, dark brown, brown pruinose, apex obtuse. The asci are subcylindric or narrow-clavate, 150–175μ long, 7μ diameter, and the free part-spores measure 16–24 × 1·5μ. By transmitted light, the wall of the perithecium is rather dark brown. The host appears to be a beetle larva, but not a Melolonthid.

I have examined another specimen, collected at Norwood, Mass., 27 August 1933, on larvae buried about one-half to one inch deep in

leaf mould and soil in woods composed chiefly of *Acer rubrum*. In this, the part-spores were cylindric, sometimes attenuated to one end, 14–27 × 1·5–2μ.

This is a *Cordyceps*, not *Ophiocordyceps*, differing from *O. acicularis* in the shape of the perithecia and the character of the ascospores. The length of the part-spores is exceptional for an entomogenous *Cordyceps*.

106. *Cordyceps subsessilis* Petch, n.sp.

It is doubtful whether this species should be referred to *Cordyceps* or to *Torrubiella*. It is represented in the Farlow Herbarium, *ex* Herb. Thaxter, by two specimens, both on a coleopterous larva in wood, No. 6145, Burbank, Tennessee, August 1896, and No. 6155, Cranberry, North Carolina, 1887.

In No. 6145, the fungus has an irregular, strap-shaped pseudostalk, 5 mm. high, 1 mm. broad, smooth, yellowish, with brown, horny patches here and there, which bears a cluster, 2 mm. diameter, of perithecia at the apex. In No. 6155, the larva has apparently been cut longitudinally and the specimen broken up; all the perithecia are now detached, and there are fragments of a thin, brown, horny clava. Judging from these specimens, it would appear that the fungus produces perithecia as soon as its mycelium reaches the surface of the wood, the apparent stalk being merely the strand of mycelium in the wood, probably in the insect bore-hole.

The perithecia are remarkably large, 1–1·5 mm. high, 0·33–0·44 mm. diameter. They are numerous, superficial, free, crowded, narrow flask-shaped, collapsing laterally, amber, glabrous, or with slight, white or yellowish mycelium between their apices. The asci are cylindric, capitate, 240 × 5μ, and the part-spores cylindric, 3–7 × 1μ.

Cordyceps subsessilis Petch, n.sp.—Stipitibus irregularibus, flavis dein brunneis, ligno immersis, apice perithecia conferta ferentibus; peritheciis liberis, anguste ampullaceis, 1–1·5 mm. alt., 0·33–0·44 mm. diam., a latere collabascentibus, succineis, glabris; ascis cylindraceis, capitatis, 240 × 5μ; articulis ascosporarum cylindraceis, 3–7 × 1μ. On larvae of Coleoptera in wood, North America.

107. *Cordyceps curculionum* (Tul.) Sacc.

Cordyceps curculionum was figured by Robin as *C. entomorrhiza*, and his mistaken identification was corrected by Tulasne. It appears to have been fairly well known in the middle of the last century, but it is not well represented in herbaria at the present day. Cooke, writing of this species in his *Vegetable Wasps and Plant Worms*, stated: "It is generally known amongst entomologists that specimens of *Curculio* are collected in tropical countries, and sent home with the collections, which have club-shaped parasites attached to them. Foreign col-

lectors, or collectors abroad, have generally a keen eye to the monetary value of such 'varieties' and are seldom satisfied with their weight in gold to part with them. The golden opportunities are perhaps rather too rare." Whatever the reason, there are few specimens in herbaria. Incidentally it may be noted that their weight in gold would not be of great value, even at current rates. The object of the present note is to call attention to a feature, probably related to *Cordyceps curculionum*, which does not appear to have been described.

In Herb. British Museum there is a specimen of a fungus on a weevil (*Helipus* sp.), collected by Bates at Tapajos, Brazil (B.M. 165). The fungus emerges from the insect along the sutures, in the form of pale brown, loose, conical tufts, up to 1·5 mm. high, 0·6 mm. diameter at the base, tapering to a more or less acute apex. These are united at their bases, or laterally coalescent in an irregular fringe. The tufts are composed of loosely interwoven hyphae, 3–4μ diameter, smooth and flexuose. There is no central core of agglutinated hyphae, and no conidiophores or conidia have been found on them. Another specimen in Herb. British Museum (No. 166), also on a weevil (*Sternechus* sp.), collected by Bates at Santarem, Brazil, is similar to the foregoing, while in Herb. Berlin there is another example, collected by Tessmann (No. 83) in Peru.

None of these specimens could be assigned to any species on their own evidence. There is, however, another specimen in Herb. British Museum (No. 175), on a weevil (*Helipus* sp.), from Guayaquil, 26 July 1871, which, in addition to these growths along the sutures, bears a perithecial clava of *Cordyceps curculionum*. It would appear, therefore, that these sterile growths are related to that species. They are not the conidial stage, which is either a *Hirsutella* or a *Hymenostilbe*, but this has not yet been seen in a fit condition for determination.

108. *Cordyceps Erotyli* Petch, n.sp.

In *Vegetable Wasps and Plant Worms*, p. 115, Cooke, writing of *Cordyceps curculionum*, stated: "It is not improbable that the coleopterous insect alluded to by Mr Gray as belonging to the family Erotylidae (the *Erotylus taeniatus* of Columbia) was attacked by a form of this same *Cordyceps*." He then quoted: "It is remarkable for producing occasionally from the head, from between the head and thorax, and from the sides of the body, a number of very slender vegetable appendages, the apex of each ending in a small tuberculated head of a yellowish colour."

There are two specimens of a *Cordyceps* on *Erotylus* sp. in the Farlow Herbarium. No. 2625 was collected by Thaxter at Verdant Vale, Trinidad, 1912–13. The fungus is on the mature beetle, lying in its burrow in wood, and the clavae emerge from the entrance to the

burrow. The insect is sparsely covered with yellowish mycelium, and the numerous clavae arise from the sutures on the upper and under surface, sometimes most from the head, sometimes most from the hinder end. The clavae are up to 15 mm. high, simple, or bifurcating above. The stalk is 0·15–0·25 mm. diameter, externally yellowish pruinose, internally subtranslucent and red-brown. The head is clavate or subcylindric, at first yellowish and sterile. The perithecia in an early stage are immersed, or partly immersed, in yellowish, loose tissue, but become superficial, amber, then brownish red, sometimes confined to one side of the head. The head is 1·5–4·5 mm. long, 0·36–0·75 mm. diameter, and the perithecia are conoid to flask-shaped, 0·4 mm. high, 0·18 mm. diameter, slightly tomentose. The asci are about 250μ long, 4μ diameter, cylindric, capitate, and the ascospores linear, 0·75μ diameter. Part-spores were not found.

Another specimen, No. 6120, Verdant Vale, Arima, Trinidad, 1913, bears two clavae like those of No. 2625. In addition, delicate strands of mycelium, white becoming brown, extend from the insect along the sides of the boring to the entrance, and thence radiate over the surface of the wood in a patch about 2 cm. diameter. These strands bear *Spicaria* conidiophores, simple, short, about 130μ high, with a stout septate stem, 6μ diameter, and a head, 25–45μ high, consisting of two or three whorls of flask-shaped phialides, 6–14 × 2–2·5μ. The conidia are cylindric, ends rounded, 3–5 × 1–1·5μ.

A third specimen, Farlow Herb. 2582, collected by Thaxter in the same locality, December 1912, is marked by him, "*Isaria* of *Cordyceps* on Erotylid; was with the *Cordyceps* on the same host." A white mycelium emerges along the sutures and spreads in strands along the legs, passing over to the wood, on which it forms a white sheet. Conidiophores occur on the mycelium and on minute, white, loose clavae up to 1·5 mm. high.

As this species does not appear to have been described, it may be known as *Cordyceps Erotyli*. It is quite unlike *C. curculionum*. The conidial stage may be named *Spicaria Erotyli*.

Cordyceps Erotyli Petch, n.sp.—Clavis numerosis, usque 15 mm. longis, simplicibus vel furcatis; stipitibus 0·15–0·25 mm. diam., externe pruinosis flavis, interne subtranslucidis, rubro-brunneis; capitibus clavatis vel cylindraceis, 1·5–4·5 mm. longis, 0·36–0·75 mm. diam.; peritheciis superficialibus, conoideis vel ampullaceis, 0·4 mm. alt., 0·18 mm. diam., confertis, interdum ab uno latere capitis deficientibus, succineis dein brunneo-rubris, leniter tomentosis; ascis cylindraceis, capitatis, 250 × 4μ; ascosporis linearibus, 0·75μ diam. On *Erotylus* sp. (Coleoptera), Trinidad.

Spicaria (*Isaria*) *Erotyli* Petch, n.sp.—Conidiophoris sparsis vel in clavis albis laxis minutis usque 1·5 mm. alt. aggregatis, simplicibus, usque 130μ alt., 6μ diam., phialidibus in verticillis duobus vel tribus

capitulum 25–45μ alt. formantibus; prophialidibus nullis; phialidibus ampullaceis, 6–14 × 2–2·5μ; conidiis cylindraceis, utrinque rotundatis, 3–5 × 1–1·5μ. On *Erotylus* sp. (Coleoptera), Trinidad.

109. *Cordyceps ramosa* Petch, n.sp.

Two collections of this species, made in Trinidad by J. B. Rorer and sent to Thaxter, are now in the Farlow Herbarium, Nos. 6133 and 2626. They were said to be on lepidopterous larvae, but that was queried by Thaxter, and the larvae appear to be coleopterous.

In each specimen, two clavae arise from the larva, which is covered with a thin layer of rufous mycelium. The clavae are up to 7 cm. high, ascending, 1 mm. diameter below, attenuated upwards, longitudinally sulcate, terete, ashy brown, glabrous, and bear up to six lateral branches which arise more or less at right angles to the main stem and curve upwards, sometimes branching again. The main stem and the branches are covered with scattered or crowded, ovate perithecia, 0·4 mm. high, 0·3 mm. diameter, glabrous, with a subacute apex. The perithecia on the branches are now dark brown, subtranslucent, collapsing laterally, while those on the main stem are rufous brown and rigid. The structure of the perithecium is the same in both situations, but only the perithecia on the branches contain asci, those on the main stem being filled with hyphae, an occurrence which is not uncommon in *Cordyceps*. The wall of the perithecium is brown, and composed exteriorly of large polygonal cells, from 9 × 7μ to 18 × 15μ. The asci are narrow clavate, 130–170 × 6–7μ, and the ascospores 1·5–2μ diameter, with septa 6–7μ apart. Neither free ascospores nor part-spores have been seen, and consequently there is a possibility that the fungus may be an *Ophiocordyceps*. In No. 2626 the perithecia are immature.

Besides its branched character, this species differs from *O. acicularis* in the nature of the main stem, the shape of the perithecia, and the length of the ascus. In general shape it resembles *Cordyceps Henleyae* Massee, but differs from that species in the shape of the perithecia.

Cordyceps ramosa Petch, n.sp.—Clavis usque 7 cm. alt., basi 1 mm. diam., sursum attenuatis, ramis lateralibus usque sex ferentibus, teretibus, cinereo-brunneis, glabris; peritheciis brunneis, superficialibus, ovatis, apice subacutis, glabris, 0·4 mm. alt., 0·3 mm. diam.; ascis angusto-clavatis, 130–170 × 6–7μ; ascosporis 1·5–2μ diam., septis 6–7μ distantibus. On coleopterous larvae, Trinidad.

110. *Cordyceps variabilis* Petch, n.sp.

This species occurs on beetle larvae in rotten wood. There are several specimens in the Farlow Herbarium, *ex* Herb. Thaxter, differing to such a degree that it is difficult to draw up a description which will cover all the forms.

In No. 613 A, on an elaterid larva, Ithaca, N.Y., 25 July 1916, coll. G. H. Duff, the clava is up to 1 cm. high. The stalk is about 0·6 mm. diameter, ochraceous, coarsely pruinose or furfuraceous. The perithecia are fused laterally in groups, 1·8–2·5 mm. long, either terminal or subterminal, or lateral at about half the height of the clava, almost or quite encircling it. The groups of perithecia are slightly pulvinate, strongly rough with the apices of the perithecia. The separate perithecia are 0·4 mm. high, 0·25 mm. diameter, flask-shaped with a conical or subcylindric apex, coarsely ochraceous pruinose almost to the apex, which is now translucent red.

In No. 6144, on a Coleopterous larva in wood, Burbank, Tennessee, 7 August 1886, the perithecia are fused in an apical plate almost surrounding the apex of the clava, which is visible only on one side. The stalk is longitudinally striate, matt, not furfuraceous, and the apices of the perithecia are conical. At the base of the clavae are white tufts of mycelium, which contain hyaline, cylindric conidia, straight or slightly curved, with obtuse ends, 10–12 × 2–2·5μ.

In three numbers, it was suggested that the fungus grew on dipterous larvae or tipulid larvae feeding in rotten wood. The hosts, however, appear to be very small Coleopterous larvae. These numbers are No. 6146, in rotten spruce log, [?] Swamp, York, Maine (no date); No. 6138, in rotten bark of *Tilia*, Kittery Point, Maine, 20 August 1920; and No. 2843, in the same locality and substratum, 1895. In these specimens, the clavae project from the wood, only to a height of about 3 mm. or less. The stalk is about 0·12 mm. diameter, pale yellow, minutely pruinose, and bears, when fertile, a small group of about four perithecia at or near the apex. The perithecia are almost free, fused together, as a rule, only at the base. Most of the clavae in these specimens bear minute acervuli, arising anywhere on the clava as white tufts and becoming subtranslucent, yellow, pulvinate, about 0·4 mm. diameter, and containing cylindric, hyaline conidia, slightly curved, 5–6 × 2μ. It is probable that these acervuli are those of a fungus parasitic on the *Cordyceps*. Evidence has been found that the conidial stage of the *Cordyceps* is a *Hirsutella*, but the specimens are not in a fit condition for description.

Two other specimens in the Farlow Herbarium, *ex* Herb. University of Wisconsin, are marked respectively "*Cordyceps* in rotten wood, Blue Mounds, Wis., August 7, 1905", and "*Cordyceps*, stroma yellowish, 0·5–1 mm. × 5–8 mm., Blue Mounds, Wis., August 10, 1906". These are small forms on beetle larvae, resembling those in Nos. 6146, 6138 and 2843. The second is accompanied by a slide which shows perithecia with a cylindrical apex, and cylindric part-spores, 5–7 × 1·5μ. In No. 6132 A, the asci are cylindric, 280–300 × 9μ, and the part-spores cylindric, 7–9 × 1·5–2μ.

This species resembles in some respects *Cordyceps Baumanniana*

P. Henn., from West Africa, which was said to be on a caterpillar. The type specimen appears to be on a beetle larva. Hennings figured the lateral head as strongly verrucose with the projecting apices of the perithecia, but he described it as punctulate, and according to my notes on the type the head resembles that of *C. dipterigena*. From *Ophiocordyceps macularis* Mains, this species differs in the shape of the perithecia and the ascospores.

Cordyceps variabilis Petch, n.sp.—Clavis usque 1 cm. alt.; stipitibus usque 0·6 mm. diam., teretibus, sursum attenuatis, ochraceis, crasse pruinosis vel furfuraceis; peritheciis in lamellis subpulvinatis verrucosis, stipitem fere cingentibus, apicalibus vel lateralibus, usque 2·5 mm. longis, aggregatis; peritheciis ampullaceis, 0·4 mm. alt., 0·25 mm. diam., fere ad apicem ochraceo-pruinosis, apice conico vel cylindrico, translucente, rubro-brunneo; ascis cylindraceis, capitatis, 280–300 × 9μ; articulis ascosporarum cylindraceis, 5–9 × 1·5–2μ. On larvae of Coleoptera, North America.

III. *Cordyceps Melolonthae* (Tul.) Sacc.

An account of this species, based on the specimens then available, was given in *Trans. Brit. Mycol. Soc.* XIX (1935), 165. Since then, further examples have been examined, and these extend the previous idea of this fungus. All the fertile specimens previously examined were more or less obese forms, similar to that figured by Hard as *Cordyceps herculea*, and some difficulty was experienced in understanding how the long, slender, barren clavae, illustrated by Cist and Fougeroux de Bondaroy, could develop into a *Cordyceps* with a stout stalk and a head about 1 cm. diameter. That difficulty has been removed by specimens from British Guiana, which show that *C. Melolonthae* can assume long, slender forms as well as the more obese forms.

The specimens referred to were collected at Vryheid, British Guiana by Dr D. H. Linder (Linder 971). They were found at the base of a palm tree, in loose elevated soil, 5 cm. of the fungus projecting. The collector's note gives the length of the stalk as 10 cm., with a diameter of 4–6 mm., and the length of the head as 2·5–3 cm., with a diameter 7–7·5 mm. A dried specimen has a stalk, 8 cm. long, 2·5 mm. diameter below, expanding to 3 mm. above, and a head, 2 cm. long, 5 mm. diameter. The head is cylindric, somewhat flattened laterally, abruptly distinct from the stalk, with a V-shaped groove at the base on each side, and sometimes slight indications of a previous longitudinal groove. The colour when fresh was "head apricot yellow to Mikado orange; stalk light buff to almost white".

In *Trans. Brit. Mycol. Soc.* XVIII (1933), I referred *Cordyceps Rickii* Lloyd to *C. martialis* Speg. That reference was certainly incorrect, for Lloyd's photograph shows the head of *C. Rickii* abruptly distinct from the stalk, whereas in *C. martialis* the stalk merges gradually into the

head. From the photograph of *C. Rickii*, the fungus is apparently on a melolonthid, and its colour when dry was Mars yellow, according to Lloyd. It would seem most probable that *C. Rickii* is *C. Melolonthae*.

As previously stated, it has been customary in North America during recent years to refer to the "White Grub" *Cordyceps* as *C. herculea* (Schw.) Sacc., on the supposition that it was identical with *Sphaeria herculea* Schw. Lloyd, however, examined the type specimen of *S. herculea*, and found that it was a Gasteromycete, *Cauloglossum transversarium* Fr. That fact was known to Ravenel many years earlier, for when he issued *Cauloglossum transversarium* in *Fung. Car. Exsicc.* v, 79, he added the synonym "*Sphaeria herculea* Schwein!", with the personal sign that he had examined the type specimen.

Coker & Couch, in *The Gasteromycetes of the Eastern United States and Canada* (1928), p. 56, remark that "The specimen labelled *Cauloglossum transversarium* in the Philadelphia Herbarium (Ravenel, *Fung. Car. Exs.* 79) is not this species but *Sphaeria herculea* Schw., according to Ravenel." That, however, is not what Ravenel meant. What he intended to convey was that it was *Cauloglossum transversarium* and also *Sphaeria herculea*, the latter name being a synonym of the first. The corresponding specimen under the same number and label in Herb. British Museum is certainly *C. transversarium*, and there are seven other gatherings by Ravenel of that species, correctly named, in the same herbarium.

Mains (*Proc. Amer. Phil. Soc.* LXXIV (1934), 265), perhaps misled by Coker & Couch's misunderstanding, has suggested that the name, *Cordyceps herculea*, should be retained for the "White Grub" fungus, on the ground of general usage. It is difficult to see how that can be done, in view of the valid prior name, *C. Melolonthae* (1865). *C. herculea*, as applied to the "White Grub" fungus, apparently dates only from Ellis and Everhart, *North American Pyrenomycetes* (1892).

It may be noted that determinations of the "White Grub" fungus as *C. Ravenelii* (Riley, *American Entomologist*, III, 137; Massee, *Ann. Bot.* IX, 34) are certainly incorrect, and were based on immature examples of *C. Melolonthae*.

112. *Ophiocordyceps caloceroides* (B. & C.) Petch.

In redescribing this species in *Trans. Brit. Mycol. Soc.* XVIII (1933), 63, it was suggested that the reason why the type specimen was collected without its host and was recorded as occurring on the ground was no doubt that the specimen had fallen to the ground and the host had disintegrated.

Further specimens have indicated that the foregoing explanation is probably erroneous. The fungus may occur on a spider which makes a cylindrical hole in the ground and lines it with silk. When attacked

by the fungus, the spider dies at the bottom of its tube, and the fungus may grow up from it along the silk sides of the tube, not in stout strands, but as a delicate mycelium, the clavae arising only near the upper edge of the tube. The fungus would thus appear to be growing on the ground, and unless it was recognized as a *Cordyceps* and the tube carefully dug up, it would be collected without any indication of attachment to a subterranean host.

These recent examples also show that *Ophiocordyceps caloceroides* does not always take the long linear form of the type specimen. One, "on a ground spider, Grand Etang, Grenada, B.W.I., 1912–13" (Thaxter), Farlow Herb. 6122, bears two clavae, with a stalk about 8 mm. high, 0·25–0·4 mm. diameter, and an irregularly ovoid head, rounded above, 1·5–2 mm. high, 1 mm. diameter, abruptly distinct from the stalk. Another, immature specimen from Pehata, Liberia, October 1926 (Linder 1093), also on a ground spider, has at the top of the tube eleven clavae, which are narrow clavate, up to 15 mm. high, 0·25 mm. diameter at the base, expanding uniformly upwards to 0·75 mm. diameter.

113. *Cordyceps cylindrica* Petch, n.sp.

This species, which occurs on a trap-door spider, was collected by Dr D. H. Linder in St Anne's Valley, Trinidad, 22 August 1923 (Linder 111). When fresh the head was pale yellow and the stalk white, but the dried specimen is black. The spider, covered with white mycelium, is fixed at the base of the tube, and the single *Cordyceps* clava arises from the spider and emerges at the side of the trap-door.

The stalk is about 2·5 cm. long, 2 mm. diameter, irregularly twisted. The head is cylindric, 1·4 cm. long, abruptly broader than the stalk, 5 mm. diameter at the base, rapidly diminishing to 3 mm. diameter, and thence uniformly cylindric to the obtuse apex. The head is minutely rough with the ostiola, which barely project, and pruinose between them. The perithecia are deeply immersed, flask-shaped with a long neck, 1·2 mm. high, 250μ diameter. The head is red-brown in section, soft and subgelatinous internally, and, judging from the grains of sand adhering to it, it is viscid when fresh. Unfortunately, no asci were found, the specimen being apparently effete. A few spores, cylindric or narrow-oval with rounded ends, 2·5–3·5 × 1μ were observed, but it is doubtful whether these were part-spores.

It seems probable that this species is related to *Isaria atypicola* Yasuda, described from specimens on a spider in Japan, which is the same shape, and is said to be light purple above, velvety, with cylindrical conidia.

Cordyceps cylindrica Petch, n.sp.—Stipite ca. 2·5 cm. alt., 2 mm. diam., irregulariter torto, albo, sicco nigro; capite cylindraceo,

1·4 cm. alt., a stipite distincto, basi 5 mm. diam., ad 3 mm. diam. mox attenuato, dein ad apicem cylindraceo, pallide flavo, sicco nigro, pruinoso, ostiolis minute scabro, interne rubro-brunneo, subgelatinoso; peritheciis immersis, ampullaceis, collo longo, usque 1·2 mm. alt., 250μ diam. Ascis et sporis non visis. On a trap-door spider, Trinidad.

114. *Cordyceps elongata* Petch, n.sp.

In the Farlow Herbarium there are four specimens of this species, *ex* Herb. Thaxter, on the larva and pupa of *Apatela americana*, No. 6134, Burbank, Tennessee, September 1887; No. 6135, Cranberry, North Carolina, 1887; No. 6137, York, Maine, August 1893; and No. 6277, York, Maine, and Cullowhee, N.C. No. 6137 is the type.

The clavae are up to 11 cm. high, from one to three arising from each host. The stalk is flexuose, longitudinally sulcate and twisted, pale brown, nearly glabrous, about 1 mm. diameter, almost equal, or expanding upwards to 2 mm. diameter. The head is from 1 to 4 cm. long, slightly thicker than the stalk, 1·1–2·2 mm. diameter, cylindric, terete, equal, or in the thicker forms slightly attenuated upwards, obtuse, or rarely with a minute apiculus, pale yellow, rough with red-brown ostiola. Sometimes the perithecial area is lacking in a narrow strip down one side of the head. The perithecia are immersed, the tissue between their apices forming a thin, continuous sheet on the dried specimens; they are scattered or crowded, ovato-conoid, 0·5 mm. high, 0·3 mm. diameter, apex subacute, wall yellow by transmitted light. The asci are 220μ long, 8μ diameter, and the ascospores 2μ diameter, with septa 4–12μ apart. Part-spores were not seen, but the ascospores are cylindric, and the fungus is probably *Cordyceps*, not *Ophiocordyceps*.

This species resembles a thin, elongated *Cordyceps militaris*. It has, however, a different stalk, and its asci and ascospores are broader than in that species. The colours described above are those of the dried specimen. From similarity of structure of the stalk, it seems probable that its conidial stage is *Hirsutella gigantea*, described in the next note.

Cordyceps elongata Petch, n.sp.—Clavis paucis, usque 11 cm. alt.; stipitibus flexuosis, longitudinaliter striatis, tortis, pallide brunneis, fere glabris, circa 1 mm. diam., aequalibus vel sursum incrassatis ad 2 mm. diam.; capitibus 1–4 cm. alt., stipite vix crassioribus, 1·1–2·2 mm. diam., cylindraceis, teretibus, pallide flavis, ostiolis rubro-brunneis asperis, apice obtuso vel rarius breviter apiculato; peritheciis immersis, sparsis vel congestis, ovato-conoideis, 0·4 mm. alt., 0·3 mm. diam., apice subacuto, pariete flavo; ascis cylindraceis, 220 × 8μ; ascosporis 2μ diam., septatis, septis 4–12μ distantibus. On pupae and larvae of *Apatela americana* (Lepidoptera), North America.

115. *Hirsutella gigantea* Petch, n.sp.

The type specimen of this species, Farlow Herb. 6126, was collected by Thaxter "Above Diana's Bath, Intervale, New Hampshire, July 1901." It is on a Lepidopterous pupa attached to a piece of wood, and bears three clavae, all of which have been broken. Two of them have regrown, but the apices are lacking in all three. In their present condition, the height is about 4 cm. They are about 0·6 mm. diameter, brown below, longitudinally sulcate, glabrous, ashy and minutely setose above. The upper part of the clava is covered by a palisade layer of clavate or cylindric cells, rounded above, 15–18 × 6–7μ, among which are *Hirsutella* phialides up to 40μ high, with a flask-shaped base, 16–20 × 8–9μ, and a long, stout sterigma, 1μ diameter. The spore cluster is lemon-shaped, 10 × 6μ, becoming globose, 10μ diameter, and the separate conidia are broadly cymbiform with obtuse tips, 9–10 × 3–4μ.

In another specimen, Farlow Herb. 6199, on a cocoon of *Apatela*, R. Thaxter, York, Maine, about fourteen clavae, now all pale brown, arise from the cocoon. These are straight below, flexuose above, with scattered lateral branches. The specimen has been pressed, and now covers an area 8·5 cm. high and 7 cm. wide, while the clavae are so entangled that it is not possible to ascertain their exact height, but some of them certainly exceed 10 cm., though their diameter at the base is only 0·5 mm.

In contrast to the foregoing specimen is one (Farlow Herb. 648), collected by Thaxter at New Hartford, Conn., 1888–9, in which the clavae are only about 2 cm. long. These are on a small pupa, about 1 cm. long.

The bases of the branched stalks in the specimen of *Stilbella ramosa* (Peck), Farlow Herb. 6142, described later in this series, exactly resemble the stalks of *Hirsutella gigantea*.

There would seem to be some possibility that this species may be *Isaria nigripes* Schw., *North American Fungi*, No. 3068. Schweinitz stated that his species grew on buried chrysalides, and was simple, half an inch high, with a somewhat slender, black, glabrous stalk up to half the height, passing into a simple, obtuse, ashy white, pulverulent head, often falcate, the conidia rather compact. But Schweinitz's type does not appear to have been re-examined, and the fungus has not been reported since.

Hirsutella gigantea Petch, n.sp.—Clavis 2–10 cm. et ultra alt., 0·5–0·6 mm. diam., simplicibus, vel supra ramosis, teretibus, saepe longitudinaliter sulcatis, infra brunneis, pruinosis dein glabris, supra cinereis, setosis; phialidibus ad 40μ alt., basi ampullacea, 16–20 × 8–9μ, sterigmate longo, crasso, 1μ diam.; conidiis late cymbiformibus,

obtusis, 9–10 × 3–4μ. On pupae of Lepidoptera (*Apatela americana*, etc.), North America.

116. *Calonectria Hirsutellae* Petch, n.sp.

In *Trans. Brit. Mycol. Soc.* XVI (1932), 226, I described *Calonectria pruinosa*, the ascigerous stage of *Hirsutella versicolor*, a common *Hirsutella* on leaf-hoppers in Ceylon. The corresponding *Hirsutella* on leaf-hoppers in the Western Hemisphere is *H. floccosa* Speare, and in the Farlow Herbarium there is a specimen, No. 6153, collected by Thaxter at Cranberry, North Carolina, August 1887, bearing the ascigerous stage, which again is a *Calonectria*.

The insect is covered with a compact, white or cream-coloured film of mycelium which sometimes spreads out in a radiating mat over the leaf. This mycelium bears *Hirsutella* conidiophores. The perithecia are semi-immersed, globose, 0·25 mm. diameter, with a short, cylindric ostiolum, 0·05 mm. high, apex obtuse. They are now dark amber, glabrous above. The asci vary from clavate, 80 × 12μ, spores biseriate above, uniseriate below, to linear clavate, 130 × 6μ, spores uniseriate. The apex of the ascus is thickened, but not with a long, truncate, solid apex as in *Calonectria pruinosa*. The asci are eight-spored, and are accompanied by ligulate, diffluent paraphyses. The ascospores are fusoid, straight or slightly curved, ends obtuse, seven-septate, 22–27 × 5–6μ.

This species differs from *C. pruinosa* in the shape of the perithecia, the apex of the ascus, and the shape of the ascospores. It may be known as *C. Hirsutellae*.

Calonectria Hirsutellae Petch, n.sp.—Mycelio albo, insectum obducente. Peritheciis semi-immersis, globosis, 0·25 mm. diam., collo brevi cylindrico, 0·05 mm. alt., apice obtuso, succineis (sicco), supra glabris; ascis octosporis, aut clavatis, sporis supra biseriatis, infra uniseriatis, 80 × 12μ, aut lineari-clavatis, sporis uniseriatis, 130 × 6μ, apice incrassato; paraphysibus ligulatis, diffluentibus; ascosporis hyalinis, fusoideis, rectis vel leniter curvatis, utrinque obtusis, septem-septatis, 22–27 × 5–6μ. On a leaf-hopper, Cranberry, North Carolina.

117. *Torrubiella paxillata* Petch, n.sp.

In the Farlow Herbarium, *ex* Herb. Thaxter, there are three gatherings of a fungus "on aphid lion", No. 6148, Cranberry, North Carolina, 1886; No. 6150, Burbank, Tennessee, 1898; and No. 6151, Cranberry, North Carolina, August 1887. An aphid lion, I am informed, is the larva of a lace-wing fly (Chrysopid, Neuroptera). The fungus is a *Torrubiella*.

The insect, or rather the debris over the insect, is covered by a

delicate, white film of mycelium, tomentose with short, erect conidiophores. The perithecia of the *Torrubiella* are flask-shaped, 0·6 mm. high, 0·25 mm. diameter, contracted towards the base into a peg, partly immersed in the loose mycelium, now amber-coloured, minutely rugose, naked above, or with scattered, white tufts of mycelium. The asci are cylindric, capitate, 280–330 × 5μ, and the ascospores linear, 1μ diameter, almost as long as the ascus, with septa 7–9μ apart. Free part-spores were not found. No. 6148 has the more nearly mature perithecia, the other two being immature.

The conidiophores are erect, usually simple, rarely branched, 3μ diameter, hyaline, septate, with the terminal segment sometimes oval and inflated to 4μ. In some the segments of the conidiophore vary in breadth, segments 1·5μ diameter alternating with segments 3μ diameter. Nearly all the upper segments are covered with lateral, obtuse, cylindric sterigmata up to 1μ high, but these are less numerous on the thinner segments and may be lacking there. The conidia are oval or pyriform, hyaline, 2–4 × 1·5μ. This conidial form is similar to *Gonatorrhodiella coccorum* Petch, from which it differs in the shape of the conidiophore, and the size and shape of the conidia.

Torrubiella paxillata Petch, n.sp.—Mycelio insectum bysso albo obducente; peritheciis ampullaceis, 0·6 mm. alt., 0·25 mm. diam., infra contractis, paxillatis, nonnihil immersis, succineis, minute rugosis, supra glabris; ascis cylindraceis, capitatis, 280–330 × 5μ; ascosporis linearibus, 1μ diam., septatis, septis 7–9μ distantibus. On the larva of a lace-wing fly (Chrysopid, Neuroptera), North America.

118. *Torrubiella gonylepticida* (Möller)

In a collection of entomogenous fungi made in the West Indies by Dr C. B. Williams, there was one on a fairly large spider, Trinidad, December 1916. The spider was fastened to a fragment of bark by white mycelium, which sparsely covered the body and legs, and here and there formed a white or cream-coloured felted crust, somewhat nodular, especially on the abdomen. Traces of a *Spicaria*, with flask-shaped, long-necked phialides, and oval conidia, 2–3 × 1·5–1·75μ, were found, but the specimen was not adequate for description.

Recently, it has been possible to examine further specimens. In one, collected by Thaxter, Maravel Valley, Trinidad, 1912–13 (Farlow Herb. 2569), the body and legs of the spider are covered by a very delicate, white film of mycelium. Another, on a large spider, collected by J. B. Rorer, Trinidad (Farlow Herb. 2586), and one on *Mygale*, collected by Thaxter, Sangre Grand, Trinidad (Farlow Herb. 2570), bear conidiophores, but are in poor condition. The best example available was collected by Thaxter, on a large spider, Maravel Valley, Trinidad, 13 February 1913 (Farlow Herb. 2559).

The fungus covers the body and legs of the spider with a delicate white film of mycelium, which then produces a dense pile of erect conidiophores over the body, and a looser growth of the same conidiophores along the legs. The covering on the body appears granular owing to the *Spicaria* heads. It is at first white, becoming cream-coloured, on the body, the looser growth on the legs appearing greyish, with cream-coloured clusters of conidia. Here and there it produces erect, cylindric or clavate, loose synnemata, up to 3 mm. high. The conidiophores are up to 360μ high, with a stalk 3μ diameter, and have two or three whorls of prophialides, or of short branches, in the uppermost 40–60μ. The prophialides are ovate, or cylindric constricted towards the middle, 5–7 × 2·5–3μ, and the phialides flask-shaped with a long neck, up to 9μ long, 2·5μ diameter below. The conidia are narrow-oval, sometimes with one end subacute, 2–5 × 1·5–3μ, or subglobose, 2–3μ diameter, catenulate. This species may be known as *S. longipes*.

Spicaria longipes Petch, n.sp.—Conidiophoris confertis, albis dein flavescentibus, usque 360μ alt., stipite 3μ diam., prophialides vel ramos breves in duobus vel tribus verticillis supra ferentibus; prophialididibus ovatis, vel cylindraceis medio constrictis, 5–7 × 2·5–3μ; phialididibus ampullaceis, 9 × 2·5μ, collo longo; conidiis anguste ovalibus, hyalinis, continuis, interdum uno fine subacutis, 2–5 × 1·5–3μ, vel subglobosis, 2–3μ diam., catenulatis. On spiders, Trinidad.

Another specimen was collected by Thaxter in Maravel Valley, Trinidad, 1912–13 (Farlow Herb. 2563), and was marked by him, "on large spider under logs, apparently perfect form of the large, white spider *Isaria*." The body of the spider in the dry state measures about 7 × 5 mm., and the spread of its legs is about 25 mm. The spider is sparsely covered with white mycelium, and in a powdery patch on the upper side of the body are densely clustered conidiophores which agree with the foregoing description.

Scattered over the body and legs of this last-mentioned specimen are the perithecia of a *Torrubiella*. These are superficial, ovato-conoid, obtuse, 0·4 mm. high, 0·27 mm. diameter, minutely rugose, with a few hyphae at the base, and sometimes white pruinose. Their colour is now dark amber. The perithecial wall bears large, oval or subglobose, hyaline cells, up to 25μ diameter, sometimes in short chains; these are external to the wall proper, which is transversely hyphal, as usual in *Torrubiella*.

This appears to be referable to *T. gonylepticida* (Möller). Möller's fungus was on a small spider, found among fragments of bark on the ground. He stated that the perithecia were 300–400μ long and had a comparatively thick plectenchymatous wall, the outer cells of which were fairly isodiametric, and projected so that the wall appeared uneven and obtusely nodulose. On the other hand, Möller also stated

that there was not the smallest trace of mycelium on the body of the spider. This latter statement, however, must be accepted with caution, and even if true it is scarcely possible that the absence of superficial mycelium would be a constant character. On some of the specimens recorded here the mycelial covering is so slight that it might be overlooked if the specimen were preserved in alcohol. The structure of the perithecial wall would appear to be a more decisive character than the presence or absence of mycelium on the host.

If this identification is correct, *Spicaria longipes* is the conidial stage of *Torrubiella gonylepticida*.

119. *Stilbella Kervillei* (Quél.) Lingelsh.

Stilbella Kervillei was described by Quélet (as *Stilbum*) from specimens on the fly, *Leria* (*Blepharoptera*) *caesia*, found in limestone caves near Rouen and Elbeuf, and it has since been found in similar caves at Maastricht on *Blepharoptera* spp. and *Scoliocentra* spp. It was recorded for England by Mr F. A. Mason in *Journal of Botany*, August 1931, pp. 205–7, having been discovered by Mr Leslie Armstrong on *Blepharoptera serrata* in the Creswell Caves, Derbyshire.

Quélet described his species as having a white, villous stalk, often branched or proliferous, a yellow, globose head, and brown mycelium on the insect. The British specimens recorded by Mr Mason had simple stalks, but otherwise agreed with Quélet's description. The insect was partly covered by a thin, discontinuous, rufous brown crust of mycelium, in which occurred dark brown, lemon-shaped bodies, resembling the spore clusters of a *Hirsutella*, but it was not possible to separate these into distinct conidia, nor to detect *Hirsutella* phialides.

With the flies which bore the *Stilbella* were others bearing long, flexuose, hair-like, brown filaments, from 2 to 6 cm. long and 0·1 mm. diameter, which were sterile and resembled the sterile clavae which occur in some species of *Hirsutella*.

In March 1934, Mr Armstrong collected more fungi on flies in Pinhole Cave, Creswell. These included branched examples of *Stilbella Kervillei*, another example of the long, thin clavae, and a single specimen which proved to be a typical *Hirsutella*.

The last-named specimen bears four *Hirsutella* clavae. These are up to 8 mm. long, 0·2 mm. diameter, simple, terete, tapering to an acute apex, rigid, ashy, slightly pruinose, fuscous internally. The phialides are up to 27μ high, with a conoid base, 4–6 × 3–4μ. The spore cluster is lemon-shaped, about 10 × 6μ, and the individual conidia are greenish hyaline, cymbiform, ends acute, 6–10 × 1·5–2μ. The insect is partly covered by a thin, rufous brown crust of mycelium, and this contains *Hirsutella* phialides, and dark brown spore clusters, identical with those found in the brown mycelium which accompanies *Stilbella Kervillei*. I propose to call this species *Hirsutella dipterigena*.

The conidial cluster of a *Hirsutella* consists of a small number of conidia, usually parallel to one another, embedded in mucilage. In some species the adherence of the conidia is only slight, and they soon separate and may be found adhering to the lower part of the sterigma or to the mycelium. In others the cohesion is greater, but the conidia separate in water. In the present species the conidia adhere strongly to one another, and the spore cluster persists as a whole and turns dark brown when old, as in *Synnematium Jonesii*.

There is an example of the long, hair-like, sterile form of this *Hirsutella* in Herb. British Museum, on *Blepharoptera serrata*, found in a "stalactite cave", Yealhampton, Devon.

Hirsutella dipterigena Petch, n.sp.—Mycelio rufo-brunneo, insectum strato tenui interrupto obducente; clavis usque 8 mm. alt., 0·2 mm. diam., simplicibus, teretibus, rigidis, cinereis, leniter pruinosis, interne fuscis; phialidibus usque 27μ alt., basi conoideo, 4–6 × 3–4μ; conidiis viridi-hyalinis, cymbiformibus, acutis, 6–10 × 1·5–2μ, in pseudosporis limoniformibus, circa 10 × 6μ, muco conglutinatis. On *Blepharoptera serrata*, Pinhole Cave, Derbyshire.

The branched examples of *Stilbella Kervillei* had a main stem up to 16 mm. long, sometimes with an acute tip, sometimes terminating in a *Stilbella* head. The lateral branches were short and simple, usually furnished with a globose head, but the branching was irregular, the laterals being sometimes scattered along the main stem and perpendicular to it, sometimes clustered, two or three together, at varying angles. On mounting the whole of one of these branched clavae, *Hirsutella* phialides were found on the main stem, showing that it was a *Hirsutella* clava. Thus, *Stilbella Kervillei* is not parasitic on the fly, but on an entomogenous fungus, *Hirsutella dipterigena*. The brown mycelium on the fly from which the simple *Stilbella* arises is that of the *Hirsutella*.

In *Bull. Soc. Nat. Sci. Buffalo*, 1 (1873), Peck described *Stilbum ramosum* as "Head subglobose, whitish or pale yellow; stem thick, smooth, branched, white above, pallid or brownish below, sometimes creeping and sending up branches at intervals; spores minute, oblong. Dead larvae of insects buried in rotten wood." In current nomenclature this would be *Stilbella ramosa*.

I have not seen the type of *S. ramosa*, but in the Farlow Herbarium there are several specimens which appear to be referable to Peck's species. No. 6142, Burbank, Tennessee, 1896, is on a larva, apparently Lepidopterous, which bears seven scattered clavae, 7·5–14 mm. high, two of which are branched. The stalk is stout, 0·2–0·5 mm. diameter, almost equal, now pale brownish, dark brown at the base, longitudinally furrowed, minutely pruinose, becoming glabrous. The head is globose or ovoid, up to 1·25 mm. diameter, yellow, waxy. The conidiophores in the head are linear, branched, and the conidia oval, oblong-oval, or pyriform, 2–3·5 × 1–1·5μ.

Another specimen, Farlow Herb. 6135, Cranberry, N.C., 1887, also on a larva, is a new species of *Cordyceps*, *C. elongata* Petch, one clava of which has examples of *Stilbella ramosa* growing on the stalk. These are 1–1·5 mm. high, with a white, terete stalk, 0·36 mm. diameter at the base, 0·12 mm. diameter above, minutely tomentose, and a yellow, ovoid head, about 0·36 mm. high, 0·3 mm. diameter. The conidia are the same as in the previous specimen. Thus, in this instance, *S. ramosa* is a simple *Stilbella*, and it is parasitic on a *Cordyceps*.

A third specimen, Farlow Herb. 646, Woodmont, Conn., September 1888, is on a small Lepidopterous pupa, which bears about ten *Stilbella* synnemata, up to 5 mm. high, with stalks 0·15 mm. diameter, white, minutely tomentose, and dark orange, subglobose heads, 0·4 mm. diameter. The conidia are oval or subpyriform, 1·5–2·5 × 1–1·5μ, or globose, 1·5μ diameter. The pupa also bears the bases of dark brown, linear clavae, two of which have regrown, and these are the clavae of *Hirsutella gigantea*. Thus, here, *Stilbella ramosa* is parasitic on a *Hirsutella*.

Comparison of No. 6142 with No. 6135 and other examples of the same *Cordyceps* shows that the apparent stalks of the *Stilbella* in No. 6142 are really the broken stalks of *Cordyceps elongata* or *Hirsutella gigantea*, only the apices and the lateral branches being the *Stilbella*, which is actually a simple synnema.

Thus, *Stilbella ramosa* (1873) is similar to *S. Kervillei* (1884) in being parasitic on a *Hirsutella* or on a *Cordyceps* of which that *Hirsutella* is the conidial stage. The differences between the American and the European *Stilbella* are slight, and I am of opinion that they are the same species. *S. Arndtii* Lingelsh., found on *Blepharoptera serrata* in caves in Silesia, is doubtless the same species.

120. *Isaria sphecophila* Ditm.

Isaria sphecophila was described and figured by Ditmar in Sturm's *Dtsch. Flora*, Abt. III, Bd. I, p. 115, pl. 57. It grew on a hornet, which lay in a cavity in a dried stem. Ditmar's figure shows fifteen linear clavae, up to 9 cm. long, springing from the insect, most of them with a knot, or irregularly thickened region, at about one-third their height from the base. Ditmar described the clavae as "medio nodosis", but it would appear that that feature was accidental, probably caused by contact of the clavae with the edge of the cavity. He also described the upper part of the clava as pilose, and figured it minutely setulose in his drawing of part of the clava enlarged. This last character has caused some difficulty or doubt in the identification of Ditmar's species. As pointed out by Speare (*Mycologia*, XII, 63), Ditmar's figure of a setulose clava suggests that his fungus was a *Hirsutella*. On the other hand, the available specimens in herbaria which would otherwise be referred to *Isaria sphecocephala* are *Hymenostilbe*.

In September 1933 I collected an "*Isaria*" on an ichneumon at Chopwell Wood, Northumberland. The clavae were small, the largest being only 1·75 mm. high, subulate, 0·4 mm. diameter at the base, 0·2 mm. diameter above, apex subacute, cream-coloured. Under a hand lens, the clava was very minutely setulose. Nevertheless, the fungus was a *Hymenostilbe*, the setulose appearance being due to projecting phialides and their attached conidia. The phialides were flask-shaped, 12 × 4μ, and the conidia clavate, the upper end rounded, the lower truncate, hyaline, smooth, 6–10 × 4–5μ.

In a specimen, Rehm, Ascomyceten, No. 1287, "*Cordyceps sphecophila* (Kl.) B. & C.", there was a conidial clava. This was not setulose under a lens. It bore a palisade layer of phialides, 16–24μ high, 4μ diameter, clavate, with a truncate apex. The conidia were clavate, 7–9 × 3·5–4μ. This conidial stage is *Hymenostilbe*.

In a specimen from Porto Rico in the Farlow Herbarium, a similar conidial clava had phialides flask-shaped, or narrow-oval, attenuated at the apex, 13–18 × 4–5μ, and conidia oval or narrow-oval, obtuse, 9–12 × 4μ.

It may be concluded that *Isaria sphecophila* Ditm. is a *Hymenostilbe*, and that it is the conidial stage of *Cordyceps sphecocephala* (Kl.) Cooke. It will stand as *Hymenostilbe sphecophila* (Ditm.).

121. *Hymenostilbe ampullifera* Petch, n.sp.

This species is included in the Farlow Herbarium under two numbers, No. 727, "on gnats, coll. R. Thaxter, Cranberry, N.C., August 1887", and No. 6254, "on gnats, coll. R. Thaxter, Cranberry, N.C., 1887", probably parts of the same gathering. No. 727 bears the identification of the insect as a tipulid, *Dicranomyia pubipennis* O.S.

The insects bear small, white, irregularly cylindric or conical clavae from the joints of the legs, the body, and the tip of the abdomen, as well as minute, white tufts scattered along the legs. The largest clava is cylindric, 3 mm. long, appearing 0·25 mm. diameter because of a loose covering of conidia, but about 80μ diameter without the conidia, and tapering upwards to about 40μ. The central core is composed of agglutinated parallel hyphae, and bears a palisade layer of flask-shaped phialides, 11–15 × 4–5μ, and barren, cylindric or clavate cells, rounded above, not verrucose, 10 × 3μ. The conidia are hyaline, narrow oval, 7–11 × 2–2·5μ, compressed and appearing cylindric, 1·5μ thick, when viewed laterally. This species differs from *Hymenostilbe muscaria* in its phialides and conidia.

Hymenostilbe ampullifera Petch, n.sp.—Clavis minutis, albis, pruinosis, usque 3 mm. alt., 80μ diam., supra attenuatis; phialidibus ampullaceis, 11–15 × 4–5μ, cellulis sterilibus, cylindraceis vel clavatis, apice rotundatis, intermixtis; conidiis anguste ovalibus, hyalinis,

7–11 × 2–2·5μ, a latere compressis et cylindraceis, 1·5μ crass. On *Dicranomyia pubipennis* O.S. (Tipulidae), North America.

122. *Hymenostilbe fragilis* Petch, n.sp.

This species, which occurs on Orthoptera, was collected by Thaxter in Verdant Vale, Trinidad, October 1912, "on an orthopterous larva" (Farlow Herb. 2581). The insect is covered with a yellowish loose film of mycelium, from which arise large numbers of white stilboid processes, erect, usually flexuose, up to 1·3 mm. high, 0·1 mm. diameter at the base, almost equal, or attenuated upwards to 0·05 mm. diameter, expanding into a clavate or ovoid head, up to 0·18 mm. diameter. Both the head and the stalk bear a palisade layer of cylindric phialides, 6–9μ high, 3μ diameter, with an obtuse, rounded apex, rough with spherical globules up to 1μ diameter. The conidia are fusoid or narrow-oval, ends obtuse, 6–10 × 2–3μ. The outer layer of the clava readily disintegrates under pressure, or when old, leaving a thin, glabrous, horny, linear clava, sometimes pruinose with the remains of the conidial layer. I have examined another specimen of this species on a locustid from British Guiana, collected by E. B. Martyn. I propose to name it *Hymenostilbe fragilis*.

A specimen collected by F. W. Urich, "on a cricket", Concord Estate, Sangre Grande, Trinidad, 4 January 1917 (Farlow Herb. 6162) bears two perithecial clavae and large numbers of conidial clavae. The latter are 0·6–0·8 mm. high, 0·02 mm. diameter below, gradually becoming clavate in the upper half and 0·06 mm. diameter above. Disintegration of the outer layer has set in, and the stalk is yellowish, translucent and horny, but the head is still entire and white. The phialides agree with those of the previous specimens, and the conidia are fusoid, sometimes oblique at the base, with a subacute apex, 8–10 × 2μ. The conidia are borne on a minute, central sterigma, not on the globular processes. The perithecial clavae in this specimen are *Cordyceps Uleana* P. Henn. Consequently, *Hymenostilbe fragilis* is the conidial stage of *Cordyceps Uleana*.

Another specimen of *C. Uleana*, which bears both perithecial and conidial clavae, is included in the cover of "*Cordyceps Sphingum*" in Herb. Kew., "Traill 23, on Orthoptera, R. Jurua, 7. 11. 74."

Hymenostilbe fragilis Petch, n.sp.—Clavis usque 1 cm. alt., 0·5 mm. diam., rectis vel curvatis, aequalibus vel sursum attenuatis, in capitulo albo clavato vel ovali desinentibus; phialidibus cylindraceis vel ovatis, 6–13 × 3–4μ, apice rotundatis, supra glandulosis; conidiis fusoideis vel clavatis vel ovalibus, obtusis vel uno fine subacutis, 5–12 × 2–3μ. On Orthoptera, Trinidad, British Guiana, Brazil.

123. *Hirsutella subulata* Petch

The only previously known specimen of this species was that on a caterpillar found at Milton, Northamptonshire, England, and recorded by Berkeley and Broome as *Isaria floccosa* Fr. in *Ann. Mag. Nat. Hist.*, Ser. 5, VII (1881), 130.

In the Farlow Herbarium, No. 4, there is a specimen on the larva of *Aegeria pyri* Harris in apple bark, coll. J. L. Zabriskie, Flatbush, L.I., N.Y., 23 September 1890. The larva bears three clavae, with the bases of four or more others. The clavae are now ashy, becoming pale brown, linear, terete, up to 1·5 cm. high, 0·3 mm. diameter, tapering upwards, minutely pruinose. The conidia measure 4–6 × 1·5–2μ, and the spore cluster is oval, up to 8 × 5μ.

Another specimen (Farlow Herb. 6241), on the larva of the "Codling Moth" under a piece of bark, was collected by Riley at Washington, D.C. The clavae are now broken, but there were apparently about a dozen, up to 7 mm. high, 0·4 mm. diameter at the base, clustered, tapering upwards to an obtuse tip, curved above, white pruinose, internally yellowish and subtranslucent. The spore cluster is oval, 6 × 3μ, and the conidia cymbiform, 4 × 1·5μ.

124. *Isaria Barberi* Giard

In *C.R. Soc. Biol.*, Paris, séance du 22 Déc. 1894, p. 823, Giard gave the name *Isaria Barberi* to a fungus on the larva of the Moth Borer (*Diatraea saccharalis* Fabr.), which had been sent to him from the West Indies by Dr C. A. Barber. The yellowish clavae arose from all parts of the larva, and were oriented in the direction of its length, probably because the fungus attacks the insect in its burrow and the clavae grow towards the entrance. The best developed clavae were like a pollarded tree in shape, with a short, basal stem, 5–15 mm. long, 1 mm. diameter, which bore at the apex two to four branches, 3–5 cm. long, 0·6 mm. diameter. The conidiophores were scattered along the clavae, and were cylindrico-conical, 4–5μ high, with one, sometimes two, slender sterigmata, 3–4μ high, terminated by an ovoid spore, 1·5μ long.

In *Ann. Bot.* IX (1895), 18, Massee described a *Cordyceps* on the same insect from Barbados under the name *C. Barberi* Giard, stating that the specimens had been sent to Kew by Mr John R. Bovell during the autumn of 1894, and had been labelled *C. Bovellii*, but the name *C. Barberi* had been adopted as having priority, on the assumption that *Isaria Barberi* Giard was the conidial form of the ascigerous condition described.

In Herb. Kew., in the cover labelled *Cordyceps Barberi*, there are three packets. One of these is marked "*Cordyceps Bovellii* Mass., on

larvae of sugar-cane borer, in sugar cane, Barbados, November 1894", and contains several larvae and clavae, the latter being linear, sometimes with short lateral branches, up to 15 mm. long, 0·25 mm. diameter, straight or variously curved, white or ashy, becoming brown, mostly detached. A second packet is marked "*Cordyceps* sterile, parasitic on insects injurious to sugar cane, Barbados (Barber)", and contains a single larva, with clavae up to 8 mm. long, 0·3 mm. broad, somewhat flattened, dark brown, smooth, matt, with short, thorn-like lateral branches. The third packet is marked "*Cordyceps Barberi* Giard, on larva of borer in sugar cane, Trinidad, Hart, October 1900", and contains apparently a pupa, which bears several short, linear or subulate clavae, white or cinereous, up to 3 mm. long.

Massee gave a figure of *C. Barberi*, but there is nothing now among the specimens which answers to the description and figure, and Dr F. J. Seaver has kindly informed me that there is no specimen of *C. Barberi* in the Massee collection in New York. The specimens in Herb. Kew. are a *Hirsutella*. The base of the phialide is ovate, conoid, or cylindric, 5–7 × 3–4μ, attenuated into a slender sterigma, 7–9μ long. The spore cluster is subglobose or oval, 4–7 × 3·5–4·5μ, and the conidia are narrow-oval or fusoid, 4·5–7 × 2μ, one end acute. On the older parts of the clava, many of the phialides are larger and irregular, with a cylindric, septate base, sometimes branched, probably due to growth after the abscission of the conidia.

Giard's description of the conidiophore is that of a *Hirsutella*. The clavae described by him were probably abnormal, their shape being perhaps due to accidental decapitation at the entrance of the boring and subsequent regrowth. The dimensions of the conidiophore and the conidia given by Giard are smaller than those obtained from the Kew specimens, but there is not much doubt that the latter belong to his species, which must now be known as *Hirsutella Barberi* (Giard).

125. *Isaria tenuipes* Peck

This species was described by Peck in the 31*st Report, New York State Museum* (1879), p. 44, as "Stem very slender, elongated, glabrous, lemon yellow, 1–1½ inches high, divided above into a few irregular branches, which are wholly covered by the white mealy coating of conidia; conidia oblong-elliptical, 4–5μ long. Dead pupae buried under fallen leaves. Center. Sept."

Pettit, in *Artificial Cultures of Entomogenous Fungi* (1895), described what he considered to be Peck's species, but as he stated that the conidia were oval to globose, 2·5–3·5μ, it would seem doubtful whether he had *Isaria tenuipes*.

I have not seen the type of *I. tenuipes*, but several examples of an *Isaria* which appears to answer to Peck's description were included in

a collection of entomogenous fungi submitted to me by the Mycological Herbarium, Cornell University. They were collected at Taughannock Gorge, New York, 3 September 1908 (No. 2906). The clavae are up to 2·5 cm. high, either simple, 0·3 mm. diameter below, with a narrow clavate head up to 1 mm. diameter, or branched above, the apices of the branches divided and flattened flabelliform, or, in the smaller forms, plumose. The colour is now white, the stalk becoming brownish in the larger specimens, but the ultimate branches have a yellow core when viewed by transmitted light. The stalk is pruinose, and the head is granular with minute spheres. These spheres are solitary at the ends of simple hyphae, 50–80μ long, 2·5μ diameter, which arise singly from the clava, or as lateral branches of long, free hyphae. The apex of the hypha (conidiophore) bears a cluster of cells, either oval, $5 \times 4\mu$, or globose, about 3μ diameter, from which arise phialides, either broadly flask-shaped, $4–5 \times 1·5–3\mu$, or subglobose, 3μ diameter, with a conical neck. The conidia are hyaline, cylindric or oblong oval, sometimes inequilateral or subcymbiform, usually straight, sometimes slightly curved, $4–6 \times 1·5–2\mu$.

This *Isaria* belongs to the *Isaria japonica* group, a type of *Isaria* which is common in the tropics. In their fully developed form, the species of this group are much branched above, but the branches are somewhat distant and distinct from one another, so that the head is plumose, contrasting with the more solid head of the *I. farinosa* type. The long simple stalks of the conidiophores also add to the feathery appearance of the head. The conidia are usually cylindric, straight or curved. Lloyd's photograph of "*Isaria farinosa*" in *Synopsis of the Cordyceps of Australasia*, fig. 613, appears to be *I. tenuipes*.

This species should be compared with *I. furcata* Schw., *Synopsis N.A. Fungi*, No. 3067, if the type of the latter is available.

126. *Isaria suffruticosa* Cke. & Massee

Isaria suffruticosa was described by Cooke and Massee in *Grevillea*, XIX (1890), 45, from a specimen on a hairy caterpillar collected at New England, Australia. Cooke gave a further account of it in *Vegetable Wasps and Plant Worms*, in which he stated that it was in tufts about 3 cm. high, and white. The stem was quite distinct, and either smooth or mealy, divided in the upper portion into very slender interwoven branches, which bore lateral branches up to the acute tips. The whole was "compacted of delicate threads, the extremities of which were free and bore the minute, ellipsoid conidia." The original description stated that the conidia are narrow-ellipsoid, $4–5 \times 1·5\mu$, borne singly at the apices of short sterigmata. A drawing which accompanies the type specimen shows a long hypha with short lateral branches bearing single conidia, but that was probably drawn from

a hypha to which conidia were merely adhering. The conidiophores on the specimen are quite different.

There is no insect in the type specimen now. It consists of a single clava, about 3 cm. high. The stalk is now brown, white pruinose, with numerous, slender, spreading branches in the uppermost centimetre. The core of the branches is yellow by transmitted light. The branches give off lateral branchlets, and both bear, as conidiophores, long, septate hyphae, 2μ diameter, which terminate in a cluster of globose cells, 3–5μ diameter, bearing globose phialides with a conical neck, 4–6 × 3 -3·5μ, the whole forming a more or less globose head. The conidia are subcymbiform, or cylindric, or oblong-oval, straight or slightly curved, hyaline, continuous, 3–6 × 1·5–2μ.

I. suffruticosa belongs to the *I. japonica* group. It may, indeed, be the same as *I. japonica*, but I have not seen an authentic specimen of the latter.

127. *Isaria Oncopterae* McAlp.

This species was described by McAlpine in *Proc. Roy. Soc. Victoria* (1894), p. 165. His account gives a few more details than are included in the description in Saccardo, *Sylloge Fungorum*, xi, 642.

The fungus occurred on the larva of *Oncoptera intricata* Walk. (Lepidoptera). "About twenty specimens were found near Melbourne, between August and October, inside the grassy tubes made by the larvae, and in every case either on a level with the surface or above it. All the infected larvae observed were nearly full-grown and dead, but in no instance were they found dead below the surface of the ground, although many tubes were examined, the larvae being always alive and apparently healthy when found below the surface." As many as fourteen clavae arose from one larva. The clavae were 1·8–3·6 cm. high, at first purple, then brown ("dirty brown root colour") with a white apex, slender, branched, velvety, bearing conidia towards the apices of the branches. The conidia were spindle-shaped to oval, hyaline, 12 × 6μ, borne on tips of hyphae at right angles to the clavae.

Apparently no specimens have reached England. From the description, the fungus appears to be a *Hymenostilbe*.

128. *Spicaria* (*Isaria*) *laxa* Petch, n.sp.

This species was collected by Thaxter, on a wireworm at Kittery Point, Maine, September 1902 (Farlow Herb. 641). The larva, which was apparently buried, bears small, white, rudimentary clavae, or strands of mycelium, from all the sutures. These clavae are fasciculate, loose, linear, up to 2 mm. long, 0·1 mm. diameter, and consist of hyphae, 4μ diameter, which bear short, lateral, oval prophialides, 6μ long, 4·5μ diameter, with a group of phialides at the apex, or short,

stout, septate conidiophores, 4μ diameter, which bear whorls of three similar prophialides and a group of three divergent prophialides at the apex. The phialides are conical or flask-shaped, 6–11 × 2–2·5μ, attenuated above into a slender sterigma. The conidia are fusoid, slightly curved, with one end acute, 5–6 × 1μ.

The structure of this species is similar to that of *Isaria dubia* Delacroix. Indeed, it scarcely differs from the latter, except in the shape of the phialides. *I. dubia*, however, is the conidial stage of *Cordyceps gracilis* and occurs on Lepidopterous larvae, whereas *Isaria laxa* occurs on Coleopterous larvae and may possibly be related to *Cordyceps stylophora*.

Spicaria (*Isaria*) *laxa* Petch, n.sp.—Clavis fasciculatis, laxis, linearibus, usque 2 mm. alt., 0·1 mm. diam., hyphis 4μ diam.; conidiophoris brevibus, crassis, 4μ diam., septatis, prophialides triverticillatos ferentibus; prophialididibus ovalibus, 6μ alt., 4·5μ diam.; phialididibus conicis vel ampullaceis, 6–11 × 2–2·5μ; conidiis fusoideis, leniter curvatis, uno fine acutis, 5–6 × 1μ. On Coleopterous larvae, North America.

129. *Spicaria velutiformis* Petch

This species, which occurs on spiders, was originally described from specimens from Ceylon. I have since examined specimens from the Hawaiian Islands, Palola Valley, Oahu, 3 April 1911 (Farlow Herb. 637) and from the Philippines, Stotsenberg, Pampanga, Luzon, 11–13 March 1923 (Farlow Herb. 6246).

130. *Synnematium Jonesii* Speare

In *Mycologia*, XII (1920), 74, Speare described a new genus, *Synnematium*, with the species *S. Jonesii*. The fungus occurred on Hemiptera, *Mezira emarginata* and *M. lobata*, in Louisiana. *Synnematium*, in most respects, resembles *Tilachlidium*. It forms erect clavae, with lateral and terminal conidiophores which bear at their apices a globule of conidia embedded in mucus. The globules on the lateral conidiophores remain separate, but those on the conidiophores at the apex of the clava fuse into a large mass. The conidia are at first hyaline, but ultimately become brown or almost black. In addition, the fungus produces globose sclerotia, 150–200μ diameter, white then brown.

Speare, no doubt because the conidia are cymbiform and embedded in mucus, compared his fungus to *Hirsutella*. This genus was originally described as a Basidiomycete, and in Saccardo, *Sylloge Fungorum*, it is still included in the Clavariaceae. Consequently in vol. XXIII of that work, *Synnematium* is also placed in the Clavariaceae. But both *Hirsutella* and *Synnematium* are Stilbaceae.

In the Farlow Herbarium, No. 6147, there is a specimen of *Synnematium Jonesii* on a beetle, *Philonthus* sp., collected by Thaxter at

Kittery Point, Maine, September 1893. The insect does not bear evidence of attachment to any substratum, but it is in a broken condition and that point cannot be determined with certainty. It bears numerous erect, stilboid synnemata, caespitose, simple, or divaricate above, with a white stalk and a black head. The stalk is about 0·5 mm. high, 40μ diameter below, expanding upwards to 80μ, and is composed of rather loose hyphae, the outer divergent and upwardly directed. The outer hyphae of the stalk are 3–4μ diameter, septate, hyaline, verrucose, with free, clavate, verrucose tips. The basal hyphae on the insect are smooth, brown, septate, 3–5μ diameter. From the stalk, long hyaline hyphae, 1μ diameter, diverge, and bear at the apex a minute globule of mucus, which contains broadly cymbiform conidia, $9 \times 4\mu$, with rounded ends, at first hyaline, becoming brown. Sometimes these lateral conidiophores are compound, i.e. the stalk consists of a small number of parallel hyphae. At the apex of the synnema, a cluster of conidiophores is produced, but all the globules of these conidiophores fuse into a depressed globose, apical mass, 90–120μ diameter and 80–100μ high, which turns black.

In lactic acid, the black heads are dark brown. They contain the same conidia as the lateral heads, irregularly arranged as in *Cephalosporium*, with some hyphae which are the entangled apices of the conidiophores. As in *Cephalosporium*, the conidia are produced apically and persist in a globule of mucus. The tenacity of the mucus in this fungus is remarkable, and it is difficult to separate the conidia. Both the conidia and the mucus turn brown. Though the conidia are, in general, fairly uniform in size, some of the globules on the lateral, simple conidiophores contain cymbiform conidia, $3–4 \times 1·5–2\mu$, or oval conidia, $3 \times 1·5\mu$.

Another specimen, Farlow Herb. 6262, on ? *Pardomis*, collected by Dr W. H. Weston Jr. at Washington, D.C. is in an earlier stage of development. The clavae bear lateral, simple conidiophores with small apical globules containing cymbiform conidia, but none of them has an apical mass of conidia. However, the larger clavae terminate in a loose apical pencil of conidiophores. In this specimen, the conidia are hyaline.

Both the specimens recorded above answer to the description of the conidial form of *Synnematium Jonesii*, but they do not bear any sclerotia. On the other hand the following specimens, which bore numerous sclerotia but no conidiophores or stilboid clavae, have been examined; on *Harpalus* (Carabidae), coll. G. F. Atkinson, Auburn, Cal., 8 August 1890 (Farlow Herb. 694); on a large leaf-hopper, coll. J. R. Johnston, Costa Rica (Farlow Herb. 6143); on *Proreus simulans*, Los Banos, Laguna, February 1932, G. O. Ocfemia; on a Membracid, *Basilides bipennis*, Njala, Sierra Leone, December 1931; on a beetle, *Promecotheca bicolor*, Fiji; on *Helopeltis*, Belgian Congo. In all

these specimens the sclerotia have the same structure. In lactic acid, when subjected to light pressure, they readily separate into their component cells, which are thick-walled, irregularly ovoid, 11–15 × 8–10μ, or subglobose, 8μ diameter.

R. L. Steyaert and J. Vrydagh have published an account of the sclerotia on *Helopeltis* in *Étude sur une maladie grave du Cotonnier provoquée par les piqûres d'Helopeltis* (1933). They state that the sclerotium is composed of two kinds of cells, the inner hyaline and irregularly angular, the outer pale brown, more globose and smaller, both kinds having very thick walls. Grown in culture on "agar saccharosé et peptoné", the sclerotium produced a dense, white mycelium, on which sclerotia were again formed. These authors give the dimensions of the sclerotia on the insect as 48–100 × 48–84μ (mean 77·6 × 89·2), and in culture, 84–140 × 84–128μ (mean 100 × 117·4). The internal cells of the sclerotia in culture measured 14–24 × 8–24μ.

Speare stated that in culture the sclerotia produced the synnemata. As Steyaert and Vrydagh failed to obtain the conidial form in culture, there seems to be some doubt whether the sclerotia on *Helopeltis* belong to *Synnematium Jonesii*. It would, however, appear advisable that all these sclerotia should be referred provisionally to that species.

131. *Aegerita insectorum* Petch, n.sp.

This species occurred on the larva of *Urophora solstitialis* L. (Diptera) in the flower heads of *Centaurea nigra* at the Entomological Field Station, Cambridge. It forms pulvinate sporodochia on the larva, but may also produce a white mycelium, bearing clusters of conidiophores, in the cavity occupied by the larva.

Aegerita insectorum Petch, n.sp.—Sporodochiis hemisphaericis, usque 0·3 mm. diam., 0·2 mm. alt., sordide albis; hyphis fasciculatis, ramosis, 4–7μ diam., aut moniliformibus, segmentis ovoideis, aut subregularibus, segmentis oblongis medio contractis, 18–20μ longis; conidiophoris irregulariter conoideis vel ampullaceis, 14–27 × 5–9μ, interdum ovalibus, 12 × 6μ, apice obtusis; conidiis hyalinis, levibus, globosis, 6–9μ diam., vel ovalibus, 9–10 × 5–8μ, apiculatis. On the larva of *Urophora solstitialis* L. (Diptera), Cambridge, England.

132. *Sporotrichum Peteloti* (Vincens) Petch

In *Trans. Brit. Mycol. Soc.* XVI (1931), 55, it was pointed out that *Beauveria Peteloti* Vincens was evidently a *Sporotrichum* parasitic on *Hirsutella Saussurei*, and in the same journal, XIX (1935), 186, another *Sporotrichum* parasitic on entomogenous fungi, *S. columnare*, was described, differing from Vincens's fungus in the size and mode of attachment of its conidia. Since then several other specimens of *Sporotrichum* parasitic on entomogenous fungi have been examined, and it

is now possible to summarise the differences between the two species. Both produce white, conical or cylindric clavae, in addition to mycelium overrunning the insect, and both have more or less geniculate conidiophores. In *S. columnare*, the conidia are oblong-oval, one end acute, or pyriform, 6–11 × 2–4μ, and are attached just above the angles of the conidiophore, sometimes in a group of two or three, as well as along the upper part of the conidiophore. In *S. Peteloti*, the conidia are smaller, oblong or oval, 3–5 × 1–2μ, and are attached singly at the angles, or along the upper part of the conidiophore.

Revision of the specimens has shown that both species were represented in the collections enumerated in *Trans. Brit. Mycol. Soc.* XIX (1935), 187, though the figure given there is that of *S. columnare*. The revised lists of hosts and localities is as follows.

Sporotrichum columnare. On a spider capsule, Maricao, 23 March 1916 (Cornell University, 730); on a spider capsule, Maraval Valley, Trinidad, coll. R. Thaxter, 18 February 1913 (Farlow Herb. 2578); on leaf-hoppers, Burbank, E. Tennessee, coll. R. Thaxter, 1896 (Farlow Herb. 6232).

Sporotrichum Peteloti. On *Hirsutella radiata* on flies, Essequibo, British Guiana, 11 November 1916 (C. B. Williams); on *H. entomophila* Pat. on beetles, Moruga, Trinidad, 14 January 1921 (W. Nowell); on *Cordyceps dipterigena*, Cranberry, N.C., July-August 1887, coll. R. Thaxter (Farlow Herb. 6221); on *C. dipterigena*, Burbank, Tennessee, 12 August 1896, coll. R. Thaxter (Farlow Herb. 6266); on a beetle, probably on *Hirsutella entomophila*, Trinidad, March 1917, coll. J. B. Rorer (Farlow Herb. 638); on razor-back hopper, Maraval Valley, Trinidad, 3 May 1913, coll. R. Thaxter (Farlow Herb. 2573).

133. *Acremonium tenuipes* Petch, nom.nov.

This species is represented in the Farlow Herbarium by two specimens, No. 728 and another unnumbered, on spiders, evidently parts of the same collection made by W. G. Farlow in a greenhouse at Cambridge, Mass., 24 January 1890.

The fungus forms a rather loose, large, white mass of mycelium, involving the body and the bases of the legs of the spider. The host was evidently lying free, unattached to any substratum. The hyphae are regular, up to 2μ diameter, and bear lateral conidiophores up to 20μ long, which are either subulate, 1μ diameter at the base, attenuated upwards, or very slender and hair-like throughout. The conidia are hyaline, oblong or oblong-oval, continuous, 2·5–5 × 1·5μ, terminal and solitary on the conidiophores. The slender conidiophores appear to break up readily, as numerous short lengths are usually present in a preparation.

I have specimens of this fungus collected in England, on a spider's

egg cluster, Reffley Wood, near King's Lynn, 8 June 1930, and on a spider attached to a living leaf, Holt House Wood, near King's Lynn, 17 August 1931. Both these are in poor condition, and have been unrecorded, awaiting better material. In the former, the conidiophores are up to 55μ long, 1μ diameter below, attenuated upwards, and the conidia measure 2–3 × 1·5μ. In the latter, the conidiophores are up to 66μ long, 2–2·5μ diameter at the base, soon attenuated to a fine thread, and the conidia measure 2·5–3 × 1·5–2μ.

Cavara, in *Fungi Longobardiae exsicc.* (1892), No. 240, issued specimens of a fungus on a spider, with a description and figure, under the name *Sporotrichum aranearum* Cav. Its mycelium was said to consist of very slender hyphae, 0·6–0·7μ diameter, and its conidia were described as ellipsoidal, 2·5–3 × 0·5μ, solitary at the apex of the branches. Cavara's figure shows a straight hypha which gives off lateral branches, some of which again bear lateral branches. These branches are about the same diameter as the main hypha at the base, but are soon attenuated into a long, slender thread. The conidia are solitary at the ends of the main hypha and the branches. The mycelium is shown covering the body of the spider in a large, cottony mass, and minute tufts emerge from the joints of the legs.

There is an example of Cavara, *Fung. Longob. exsicc.*, No. 240, in Herb. British Museum. The hyphae are 1·5–2μ diameter, and bear the same conidiophores as described above for the American and British specimens. Cavara's measurement of the mycelium evidently refers only to the thinner part of the conidiophore. The conidia are oblong or oval, 2–5 × 1–1·5μ. Perhaps a dropped figure in printing accounts for Cavara's smaller breadth. There is no doubt that the fungus is the same as the American and British species.

Sporotrichum aranearum is recorded by Ferraris, *Flor. Ital. Crypt.* 1, from Pavia, Sicily, and Verrua (Piedmont), while Lindau, *Rabh. Krypt.-Flora*, recorded it from Muskau in Silesia on the authority of Sydow.

Sartory, Sartory and Meyer, in *C.R. Soc. Biol.*, Paris, CVII, 14 (1931), pp. 53–55, gave an account of an unnamed species of *Verticillium*, which had been found in association with a *Sporotrichum* on spiders. Their description is from cultures. The conidiophores were said to be "droits", with several verticils of primary branches, 2–4μ diameter. The uppermost primary branches were opposite. The lower primary branches bore verticils of secondary branches, 0·5–1·25μ diameter. The branches bore apical, ovoid conidia, 3·5 × 1·5μ, usually solitary, but sometimes in a group of three.

It seems most probable that this last-named fungus is the same as Cavara's. The slender dimensions of what are termed "secondary branches" and the size of the conidia point to that conclusion. But neither in Cavara's specimen, nor in the American and British ex-

amples, are the branches in verticils. They arise at irregular distances along either side of the hypha, as figured by Cavara.

As will be evident, the classification of this fungus has presented some difficulty. All observers are agreed that the conidia are usually solitary at the apex of the conidiophore or phialide, and consequently it cannot be placed in *Sporotrichum*. In this genus, the conidia are borne successively along the hypha, and though only the terminal conidium may be present, the scars of attachment of its predecessors persist.

As regards *Verticillium*, the alleged branches are not verticillate. Moreover, I have not observed erect, branched conidiophores in any specimen. Whether Sartory, Sartory and Meyer's statement that the conidiophores are "droits", means erect or straight is uncertain. The hyphae which bear the slender conidiophores or branches are certainly straight, but they are only part of the general mass of mycelium. The correct interpretation appears to be that the attenuated branches are simple conidiophores, lateral on repent mycelium. If so, the fungus is an *Acremonium*, and as the name *A. aranearum* is already occupied, I propose to call it *A. tenuipes*.

Acremonium tenuipes Petch, nom.nov.; *Sporotrichum aranearum* Cav., *Fung. Longob. exsicc.*, No. 240; *Verticillium* sp., Sartory, Sartory and Meyer in *C.R. Soc. Biol.*, Paris, CVII (1931), 53–55.—Mycelio albo, corpus et proximos articulos artuum pulvino albo tegente; hyphis regularibus, usque 2μ diam.; conidiophoris lateralibus, usque 66μ long., subulatis, basi 1–2·5μ diam., mox attenuatis, vel ab initio capillaribus; conidiis hyalinis, continuis, oblongis vel oblongo-ovalibus, 2–5 × 1–2μ, apicalibus, solitariis. On spiders, Italy, Germany, France, England, North America.

134. *Isaria brachiata* (Batsch) Schum.

This species is usually found on decaying fungi, though Saccardo, *Sylloge Fungorum*, IV, 589, added that it had been recorded on roots and leaves. Its colour is generally given as white. In September 1934 I gathered a specimen at Ingleton, Yorks., on a small mass of dead leaves; the main stem was pale brown. In October of the same year I found another specimen on a decaying agaric at West Runton, Norfolk, and in this the main stem was pale yellow when dry. The conidia in both specimens were oblong or narrow-oval, 3–6 × 1μ. In Saccardo (*loc. cit.*) the conidia are given as ellipsoid, 3–4 × 1·5–2μ. *Isaria brachiata* has *Cephalosporium* conidiophores and is a *Tilachlidium*. The type species of the latter genus, *T. pinnatum* Preuss, judging from the description, does not appear to differ from *Isaria brachiata*.

In the Farlow Herbarium, No. 759, there is a specimen of *I. brachiata* collected by Thaxter on an agaric, Cranberry, North Carolina, 1896. The conidia are oblong, 2–4 × 0·75–1μ.

The object of the present note is to call attention to another mode of occurrence of *I. brachiata*. In the Farlow Herbarium there are several specimens in which *I. brachiata* is parasitic on entomogenous fungi. Sometimes the *Isaria* may arise directly from an insect which was probably first attacked by an entomogenous fungus, or it may appear on a *Cordyceps* or *Hirsutella* clava, sometimes covering it with a continuous conidial layer, so that the whole appears to be a conidial *Cordyceps* clava.

In No. 6234, on a leaf-hopper, collected by Thaxter at Burbank, East Tennessee, August 1896, the insect bears numerous clavae from all parts of the body. These are erect or repent, up to 1·5 cm. high, 0·15 mm. diameter, usually curved, linear, with a few short, lateral, perpendicular branches. They are covered with conidiophores, which are those of *Isaria brachiata*, with *Cephalosporium* heads containing narrow-oval or subcymbiform conidia, $2–4 \times 0{\cdot}75–1\mu$. In No. 666, similar clavae, scattered or fasciculate, up to 8 mm. long, 0·25 mm. diameter, occur on a specimen of *Ophiocordyceps unilateralis*, collected by Thaxter at Kittery Point, Maine. No. 6227, "on Bombycid", collected by Thaxter at Burbank, 1887, contains a cocoon, apparently of *Apatela americana*, which bears two clavae, up to 3 cm. high, probably of *Hirsutella gigantea*, but these are covered by a uniform layer of conidiophores of *Isaria brachiata*. No. 6238, on Coleopterous larvae, collected by Thaxter at Burbank in 1896, contains two larvae, each bearing several immature *Cordyceps* clavae, up to 2·5 cm. high, which, in general, are linear, sometimes flattened above, and vary in colour from pale yellow to dark brown, but most of them have small white branches, closely adpressed to the clava, or small white tufts of mycelium, which give the clavae a mottled appearance; these small branches and tufts of mycelium are *Isaria brachiata*, though only one conidium was observed at the apex of the conidiophore in No. 6238.

I. intricata Fr. is said to differ from *I. brachiata* in having only a single conidium at the apex of the conidiophore. As the cluster of conidia in a *Cephalosporium* must begin with a single conidium, it is questionable whether that is a valid difference, if the structure of the conidiophore is the same. A specimen of *Isaria intricata* from Glamis, in Herb. Kew. *ex* Herb. Berkeley, has *Cephalosporium* heads, while a specimen of *Isaria brachiata*, Romell, *Fungi exsicc. praes. Scand.*, has some conidiophores with *Cephalosporium* heads and others with single apical conidia. On the evidence of these specimens, the two species are the same.

A REINVESTIGATION INTO THE CAUSE OF "BROWN OAK", *FISTULINA HEPATICA* (HUDS.) FR.

By K. T. St G. CARTWRIGHT, M.A., F.L.S.

(With Plates I–VII and 6 Text-figures)

The heartwood of English oak (*Quercus pedunculata* Ehrh. and *Q. sessiliflora* Salisb.) is well known for its durability and for the beautiful figure which it gives when cut on the quarter.

The colour of the heartwood varies from a light brown to a deeper and warmer shade, but occasionally assumes a rich reddish brown (*Argus brown**) in some individual trees when it is known as "brown oak" (Pl. I, fig. 1 A). This abnormal coloration may be evenly distributed throughout the heartwood or may occur in bands running parallel to the grain, when it is often known as the tortoiseshell variety, or in the West Country as "lion oak".

"Brown oak" is much prized for use in decorative work, and therefore such timber in the past has commanded a high price.

As far as is known to me, no certain means of ascertaining whether the heartwood of a standing tree will show this coloration has been discovered, up to the present, except by taking trial borings with an auger.

A similar abnormal coloration of the heartwood occurs, though more rarely, in sweet chestnut (*Castanea sativa* Mill.) (Pl. I, fig. 2 A).

Historical

No investigation into the cause of "brown oak" had been published prior to Groom's paper(1), in which he concluded that:

(1) The abnormal coloration was due to the presence of a substance which gave the reactions of the ill-defined material termed "wound gum" or "wood gum".

(2) The production of this was due to a fungus which produced conidiophores of the *Penicillium* type and was able to utilize the tannin present in the heartwood.

(3) On certain specimens small sphaeroidal basidiocarps were produced which were identified by G. Massee as those of *Melanogaster variegatus* Tul. var. *Broomianus* Berk.; no connexion between the *Penicillium*-like fungus and the latter was, however, established.

* Colours printed in italics are from Ridgway's *Color Standards and Color Nomenclature*.

(4) The fungus was stated to cause a depletion in the tannin content.

The restriction of "brown oak" to the heartwood and the distribution of the dark coloration in the streaky type made it seem unlikely that a fungus of the *Penicillium* type could be responsible. On the other hand, while *Melanogaster variegatus* var. *Broomianus* might be suspected of having some mycorrhizal association, it was unlikely to be found in the heartwood of a tree. Therefore it was decided to make a further investigation into the cause of "brown oak".

Material

Specimens of "brown oak" were obtained during 1933 and the early part of 1934 from various sources.

(1) A 1 in. board in the green condition stated to have been cut from a tree grown in Sussex in the vicinity of Lewes.

(2) From Kimberley Park, near Norwich.

(3) Two small samples from the Dartington Hall sawmills:

(*a*) In this specimen the brown coloration was evenly distributed throughout.

(*b*) "Lion oak" showing distribution in longitudinal streaks.

(4) Hedgerow tree of *Quercus sessiliflora* Salisb. from Gooseacre Farm, Radley, near Abingdon, Berks.

Examination

Microtome sections were cut from the specimens, these being examined both in the unstained and stained condition. Unless otherwise stated, sections were stained by the picro aniline blue and safranin method (2). Microscopic examination of unstained sections showed an abundance of golden to reddish brown material often in the form of globules, distributed mainly in the medullary rays and wood parenchyma (Pl. II, figs. 1, 2). Fungus hyphae could be observed with difficulty and did not appear to be abundant; their presence was usually associated with the discoloration (Pl. II, figs. 1, 2). In normal coloured wood no fungus could be detected as a rule. In stained sections it was possible to observe the distribution of the hyphae, although in the areas which were deeply coloured the hyphae remained unstained and could be detected only with difficulty. Thus there appears to be a definite association between the presence of mycelium and coloured heartwood. The hyphae are of very varied diameter, the large ones often being a deep honey colour, though some of the hyphae in the deeply coloured wood-parenchyma cells appear to lose their contents, thus showing in outline behind the dark contents of the cells (Pl. II, fig. 1). The finer active mycelium branches profusely, most frequently at right angles to the parent

hypha. These branches send out finer branches until in places a regular network of mycelium may be present. The fungus passes from cell to cell, mainly through the pits, but direct penetration through the cell wall also takes place, as is shown in Text-fig. 1. Secondary spores are rarely formed; they are usually terminal but may be intercalary.

In addition to the profuse branching, the hyphae may describe loops. Clamp connexions, which are of the simple kind, are not abundant (Text-fig. 1). On staining sections of the wood with ferric chloride, the coloured areas assume a deep blackish blue tint, showing the presence of abundant tannin; but some of the reddish brown material does not assume this colour. Tests for the so-called "wound gum" gave no very definite results. The fact that the hyphae were frequently found investing the tannin, and that sometimes the tannin appeared to have become partially broken down and lighter in colour, suggests that the fungus obtains some of its nutritive material (presumably sugar) from this source. The so-called "wound gum", which does not give a tannin reaction, may be either an excretion product of the fungus or due to a direct degradation of the wood. More direct evidence for this has been obtained, and will be found on p. 77–82. Oil-like globules, which may be colourless or pale golden to reddish, are often present on the hyphal walls.

Cultures

Cultures were obtained from all the specimens by means of a Pressler increment borer, plugs of wood being removed from the coloured areas. The outer portion of the plug was removed with a sterilized scalpel and the remainder partially buried in the medium. The medium on which the fungus was found to develop best was a 5 per cent Keppler's malt agar which had been slightly acidified with malic acid; good growth was also obtained on 5 per cent malt agar. Development was also fairly good on a 2 per cent prune agar, but on 2 per cent malt agar growth was slow and weak.

Growth was obtained from nearly all the plugs taken from the brown areas, though usually one to three weeks elapsed before it was evident, the length of time depending to a large extent on the period elapsing between felling of the tree and culturing; the fungus soon loses its vitality in the felled timber.

A few brown dusty cultures forming *Penicillium*-like conidiophores were obtained. This fungus was identified as one of the *P. divaricatum* group and is the same as that which has been obtained from oak timber showing yellow stain; it is probably synonymous with *Eidamia catenulata* Horne & Williamson, the fungus shown by Williamson (3) to be the cause of "golden oak". Cultures have been submitted to

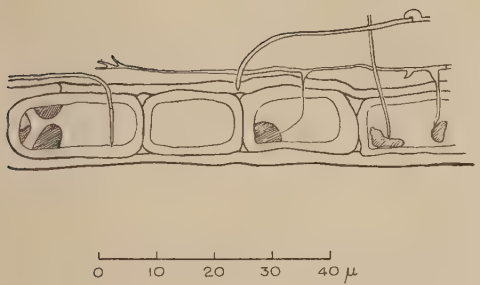


Fig. 1.

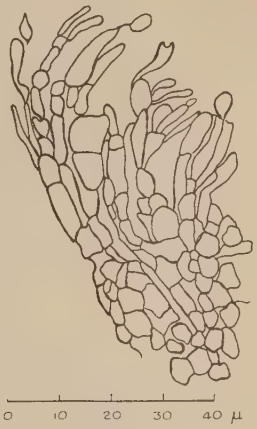


Fig. 4.

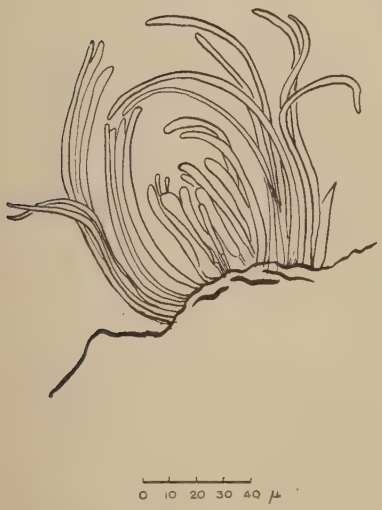


Fig. 2.

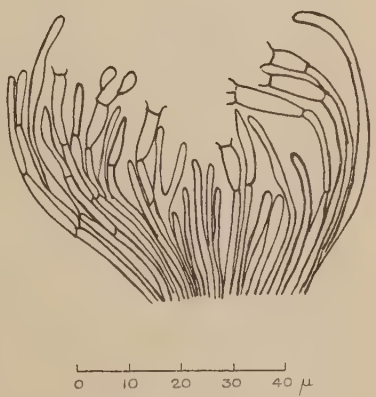


Fig. 3.

Text-fig. 1. Portion of medullary ray cell in radial section illustrating penetration of hyphae through the walls. Note also tannin masses and a single simple clamp connexion.

Text-figs. 2 and 3. Section through portion of tongue-shaped sporophore developed on 5 per cent malt agar, isolated from "brown oak" in different stages of development.

Text-fig. 4. Ditto. One side of tube showing portion of hymenial surface in detail.

Mr E. W. Mason of the Imperial Mycological Institute, who confirms that it is *Penicillium divaricatum*, or one of this group; this, C. Thom (4) states, is synonymous with *Poeciliomyces Varioti*. It appears probable that this was the fungus which Groom isolated and considered to be the cause of "brown oak". The mycelium shows no resemblance to that observed in sections of "brown oak" and, moreover, the prevalence of this fungus in all kinds of timber being second only in frequency to that of *Trichoderma lignorum* and *Penicillium "glaucum"*, should ensure abundant "brown oak" if it were indeed the cause. Oakwood inoculated with this fungus becomes stained a golden yellow, but never assumes the deep brown tints typical of "brown oak".

Description of culture

At the beginning of growth, nearly colourless coarse mycelial threads issue from the wood plug. This mycelium forms a somewhat sparse tuft suggesting the early stage of such a mould as *Mucor* except for its very slow rate of development. In a few days this nearly colourless mycelium spreads on to the surface of the medium as a thin layer in which the individual threads can still be distinguished; as it becomes denser it becomes white. Growth is always slow, and the mat at this period resembles the texture of loose cotton-wool. Growth is more rapid in the dark; the optimum temperature lies between 25 and 27° C.

The centre of the mat is usually slightly depressed and slightly zoned, but the extent of this is variable. Some cultures remain white for several weeks, but usually the mat becomes *pale pinkish cinnamon* to *pale congo pink*, the centre being *straw yellow* which deepens to *mustard yellow*, the growing edge of the culture remaining colourless. Great variation in the colour development is shown in individual cultures even in a series of plates made from the same isolation, the range shading from almost white to *Mikado orange*, *orange-cinnamon*, *light vinaceous-fawn* and *vinaceous-fawn*, in addition to the pink shades given above. The coloration is shaded into zones, and tints of russet are often apparent. Pl. III shows a typical plate culture reproduced from a hand-coloured photograph and gives an accurate rendering of the main colour range. In spite of this wide variation the tints all lie in the same general range, and the whole appearance of the cultures renders identification easy and independent of microscopic examination.

In active cultures, drops of liquid are produced from the hyphae; these drops may be colourless when produced in cultures before colour has developed in the mycelium, but they are usually produced after coloration has developed; they are then pale amber to orange reddish or liver colour, resembling the juice abundant in an active

sporophore of *Fistulina hepatica*. These drops are acid, having a *p*H value of about 3·7. Secondary spores are usually formed in abundance on culture media.

In certain cultures, as they mature, sclerotium-like lumps are produced, these usually arising in the centre of the culture at the point of inoculation. Coloured drops similar to those on the mycelium are produced from these, and sometimes small tubes or plates indicating that these structures represent rudimentary sporophores (Pl. IV, fig. 1). Under the microscope, basidia, bearing spores averaging $5 \times 3\mu$, have been observed; their formation is not confined to places where tube formation has commenced, instances having been found where the basidia are formed directly from the mycelium of the sclerotium-like lump.

In addition, tongue-shaped outgrowths are often produced, usually occurring in cultures grown in the dark. These may develop in as little as three days, in this respect their growth being more comparable with that of an agaric than of any *Polyporus* species so far cultured. Light appears to have a greater retarding action on the growth of the culture than is usual. The upper portion of these tongue-like structures is slightly swollen, so that they resemble a small species of *Phallus* in shape. On the swollen head rosette-like spots develop, this portion appearing like a zoophyte colony (Pl. IV, fig. 2). Sections show that the rosettes represent initial stages of tube formation, a normal hymenial surface bearing basidia and spores being formed. Two stages in tube development are shown in Text-figs. 2, 3, 4. The central portion upon which the tubes develop is composed mainly of large, loosely woven together conducting hyphae (Text-fig. 5).

Identification

The culture isolated from the sample plank of "brown oak" obtained from near Lewes, Sussex, did not lead to immediate identification, as at that time the wide variation in colour which occurs in different cultures was not fully appreciated. Microscopic examination of the mycelium revealed, however, the formation of secondary spores varying from 2 to 6μ or more in diameter. These were borne mostly on the aerial mycelium both terminal and intercalary, but were also observed more sparingly on the submerged hyphae (Text-fig. 6 and Pl. V, fig. 4).

The general appearance of the culture, and in particular its slow rate of growth, combined with the formation of secondary spores, suggested that the fungus isolated from the Lewes "brown oak" was *Fistulina hepatica*. Accordingly, comparative cultures were set up against a culture of *F. hepatica* already in the Forest Products Research Laboratory collection, obtained from sporophore tissue; the two were found to be identical. Further specimens of "brown oak" were

obtained from Kimberley Park, near Norwich, and in October 1933 two small specimens were obtained from the sawmills at Dartington Hall, near Totnes, South Devon. From these three specimens *F. hepatica* was also isolated from approximately 90 per cent of the plugs taken. Substantial evidence was thus obtained that *F. hepatica* is associated with the formation of "brown oak".

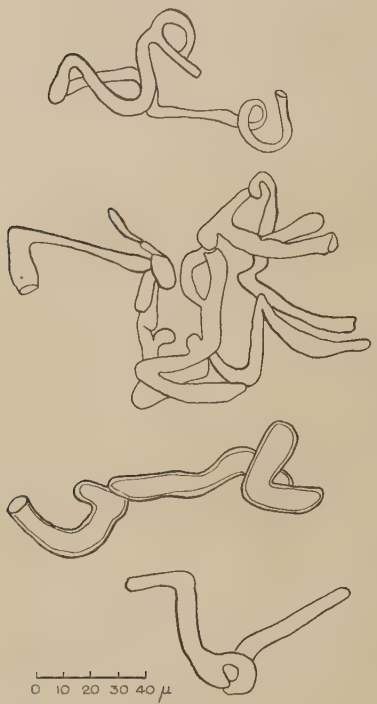


Fig. 5.

Fig. 6.

Text-fig. 5. Conducting hyphae from central portion of the same sporophore as figs. 2, 3 and 4.
Text-fig. 6. *Conidia* formed terminally on aerial hyphae.

The fact that the brown coloration is said to occur usually towards the base of a tree or near the insertion of large branches, further supported the view that the fungus responsible for "brown oak" was of the heart-rot type, gaining entry through wounds occasioned by the fall of a branch old enough to expose heartwood, or through basal

wounds. The occurrence of "brown oak" is said to be more prevalent in mature trees, and this also falls into line with the above.

Conversion of sapwood into heartwood does not usually occur in oak until between the ages of twenty-five and forty years, according to conditions of growth, but at this period there would be only a small proportion of heartwood available, therefore the chance of exposure and consequent infection would be correspondingly less than in an older tree with a larger proportion of heartwood. The occurrence of "brown oak" in comparatively young trees, though it is rare, has occasionally been reported.

In addition to the evidence set forth above, *F. hepatica* is known to cause a red rot of oak timber, termed by the French "*pourriture rouge dure*" (5).

Blocks of oak sapwood and heartwood, sterilized by steaming on three consecutive days, were placed on an active culture of *F. hepatica* grown on malt-agar medium in special modified Kolle flasks. After six months they were removed from the flasks and samples of heartwood and sapwood from one flask examined. The wood had darkened and was distinctly of a richer colour than corresponding blocks which had been steamed but not exposed to fungus attack. Stained microtome sections showed a certain amount of mycelium (mostly confined to the large vessels) in the sapwood. In sections taken from the heartwood there was abundant fungus mycelium distributed throughout, and a certain amount of brown amorphous material was also observed. These experiments were somewhat inconclusive, and it is thought probable that, as in many of the heart rots affecting the standing tree, conditions required for the rather specialized metabolism of this fungus are not easily reproduced under laboratory conditions. Living trees have been infected with *F. hepatica*, and it will be possible to follow in them the development and rate of progress in the formation of "brown oak".

Inoculation experiments

Inoculations were made by the removal of a plug of wood from an oak branch reaching into the heartwood and replacing it with one of "brown oak" containing *F. hepatica*. These inoculations were carried out in March 1934. The branch was amputated in November 1934, and the portion round the point of inoculation examined. Discoloration had taken place in both the sapwood and heartwood surrounding the inoculum, and mycelium of *F. hepatica* was observed in the wood at a distance of about 5 cm. from the inoculum (Pl. V). Some discoloration of the sapwood was probably caused by wounding and seepage of colouring matter, but the coloration in the heartwood resembled that of "brown oak".

It is proposed to follow the rate of progress of the fungus in relation to the spread of brown discoloration, but it will be some years before the results of the field experiments are completed.

Although the evidence appeared overwhelmingly to support the view that *F. hepatica* was the fungus responsible for "brown oak" formation, the failure to reproduce it in the laboratory made it necessary to apply a practical test. Accordingly, search was made for a suitable oak tree attacked by *F. hepatica*. This fungus is commonly observed on decrepit park trees which are often "stag-headed", and often almost completely hollow; but such a tree would obviously be unsuitable for selection, as complications due to the presence of other fungi able to cause decay would render any evidence obtained inconclusive.

A tree which appeared suitable for the purpose was found on the Radley College Estate, growing in a hedgerow. During the previous autumn a fruit body of *Fistulina* had been observed growing from the base of the tree. The tree, which was about ninety years old, although not a fine specimen, did not appear to be hollow, though some of the upper branches had been lost and it was slightly "stag-headed". Plugs were removed with a Pressler increment borer about two feet above the point at which the remains of the old fruit body could still be observed. The borings showed that the wood in this region was hard and was dark coloured. These plugs were placed in tubes of medium and sections were also cut from them. Sections showed the typical appearance of "brown oak" and *Fistulina* developed from the plugs placed in the culture tubes, thus showing that the fungus had spread at any rate sufficiently in the heartwood to give the desired information.

After this preliminary survey, the Radley College authorities were approached, and kindly gave me the tree. The tree was felled on 23 March 1934, being cut approximately one foot above the position of the fruit body. As felling proceeded and the heartwood became exposed, it was seen to be a rich reddish brown (Pl. V, fig. 3). The heartwood across the diameter was a uniform red-brown and this coloration extended up to the first branching and into the branches for a short distance where it began to run out into streaks as in "lion oak" (Pl. VI). The colour here was not so intense as in the main bole, tending to lessen in intensity after the first fork at eight feet from the butt end. The heartwood and sapwood appeared hard and sound throughout except where decay had entered through a dead branch, but this cone of decay did not proceed far and was not caused by *F. hepatica*. The "brown oak" was clearly defined in all the lower portion up to the first fork, extending right to the margin of the newly formed heartwood; beyond this a narrow band of heartwood of the normal colour could be detected until finally the colour ran

out in streaks. Beyond the area of brown in the portion where some of the heartwood was unaffected, a faint lilac colour could be detected in the wood adjoining the "brown oak", this being an area which the fungus was just invading. From the distribution of the "brown oak", it appears certain that infection originated through a wound at the base of the tree. As no figures are available for the rate of spread in a living tree, it is not possible to state with precision at what age the tree became infected. The slow growth of the fungus in the laboratory gives no reason for assuming that it grows at a similar rate in the standing tree. The extent and evenness of the coloration suggests that infection took place at an early stage when the tree was between thirty to fifty years old and that the fungus kept pace more or less with the conversion of sapwood into heartwood. The old scar marking the place of attachment of the sporophore suggests that this was the point of infection, and therefore it is possible that the spread of the fungus throughout the heartwood is comparatively rapid. Further data on this will be obtainable only when the results of inoculation experiments are available.

During the course of this investigation, samples of sweet chestnut were received for examination; these samples showed brown bands in the heartwood similar to those seen in the "brown oak" (Pl. IV, fig. 3). Sections showed the presence of fungus mycelium similar in appearance to that of *Fistulina* (Pl. IV, fig. 4). Cultures were made from this material and *Fistulina* obtained from it, thus showing that the fungus acts in a similar way in both oak and sweet chestnut.

Physiology

Tannin experiments

With a view to ascertaining whether *F. hepatica* is capable of utilizing tannin, an extract of oakwood which contained approximately 25 per cent tannin was obtained through the courtesy of the Forestal Land, Timber and Railways Co., Ltd. A series of concentrations of this extract was made up with 5 per cent Keppler's malt extract and 2 per cent agar; at the same time a similar series, omitting the malt extract, was also made up. In the series with malt, two plates containing 5 per cent malt and 2 per cent agar were included for comparison, as growth on this medium had been found to be excellent. In the series with tannin and agar but no malt, two plates with agar only were included. There were two plates at each concentration of tannin. The pH of the medium was difficult to arrive at by the colorimetric method, but lay between 3 and 4 in each, the addition of the tannin extract having little effect, though tending to

increase acidity. The results, averaged for each set of two plates, are summarized below:

Medium 2 % agar:

% tannin	0,	0·13,	0·25,	0·50,	0·75,	1·0,	1·25
Growth in mm. per diem	0,	2·8,	3·5,	3·6,	4·0,	4·1,	medium would not set

Medium 5 % malt and 2 % agar:

% tanin	0,	0·13,	0·25,	0·50,	0·75,	1·0,	1·25
Growth in mm. per diem	3·9,	3·9,	3·8,	4·2,	4·2,	4·2,	4·4

These results tend to show that *Fistulina* grows slightly better with the addition of oakwood-tannin extract and is able to obtain some of its nourishment from it, but as the tannin extract was not chemically pure, it does not prove that the fungus is necessarily breaking down the tannin. Further work on these lines is in progress. Pl. VII shows the stimulus to growth caused by the addition of tannin extract.

Attempts have been made to grow the fungus on a liquid tannin extract without agar, but although growth has been satisfactory for a time, it has not been considered sufficient to warrant chemical analysis of the medium before and after growth. From the fact that analyses of "brown oak" do not show much depletion in tannin and from the slowness of the action of the fungus on the mechanical strength, it is tentatively suggested that a degradation of the lignin and hemicellulose is taking place, leaving a brown gummy material as a degradation product.

As a matter of interest the following fungi were grown for comparison on the medium on which good growth was obtained with *F. hepatica*, namely 1 per cent tannin, 5 per cent malt and 2 per cent agar and using 5 per cent malt and 2 per cent agar as a control, repeating at the same time with *F. hepatica*. The results of the experiment are tabulated below:

	1 % tannin, 5 % malt and 2 % agar mm. per diem	No tannin mm. per diem
Merulius lacrymans	No growth	21·3
Polystictus versicolor	3·0	13·0
Daedalea quercina	1·5	3·8
Polyporus sulphureus	13·0	9·6
Fistulina hepatica	4·6	3·9

It will be seen that, apart from *Fistulina hepatica*, the only fungus of those tested showing stimulation of growth on the addition of the tannin extract is *Polyporus sulphureus*, which also occurs in the heartwood of oak. The effect of 1 per cent tannin on the other fungi is markedly to retard growth, as is illustrated in Pl. V, fig. 1, of *Polystictus versicolor* showing abnormal growth in a Petri dish. It is of interest to note that *Daedalea quercina* is a sapwood fungus. Growth was entirely

checked in *Merulius lacrymans* and this agrees with the fact that this fungus rarely grows on oak heartwood primarily, although under very bad conditions it can attack it.

Temperature and rate of growth

A series of Petri dishes using a 5 per cent malt agar with the addition of a small quantity of acid was inoculated from an active culture of *Fistulina hepatica*. The daily rate of increase in diameter at the different temperatures was measured, and the average daily rate of growth in millimetres at the different temperatures plotted. The figures were based on the average of ten plates. The results show that the fungus will grow over a wide range of temperature, growing appreciably at 9° C. and the optimum lying between 25 and 27° C. Growth dropped off rapidly above 30° C.

The effect of Fistulina hepatica *on the mechanical strength of oak*

It has been considered in the timber trade generally that "brown oak" is as strong as normal oak, although it is said that the "brown oak" is "milder". There was no reason to question this opinion prior to the present mycological investigation, and the results of Groom's work, in which he attributed the brown colour to the action of a mould type of fungus, strengthened this view.

The discovery that *F. hepatica* was the causal fungus suggested that mechanical tests should be carried out, as the Basidiomycetes are the group to which most of the active wood-destroying fungi belong, and in addition *Fistulina* is known to cause a heart rot of oak timber although, even in the late stages of decay, the wood never crumbles to a powder in the hand, as does wood attacked by a fungus such as *Polyporus sulphureus*. Accordingly the Timber Mechanics Section of the Forest Products Research Laboratory were asked to carry out mechanical tests.

Tests on a few small samples carried out at an early stage in the investigation had shown that there was apparently no appreciable loss in strength in the "brown oak", but as the history of these samples was imperfectly known and only a small amount of material was available, no conclusions could be based on them. More adequate material was available from the tree obtained from Gooseacre Farm, Radley, near Abingdon, although even here normal oak heartwood from the same samples could not be obtained in sufficient quantity for comparison, so that the results had to be compared with those from oak of the same specific gravity and correction made to allow for differences. The mechanical tests which were carried out were as follows

(1) Static bending test, which gives the normal strength and stiffness of timber under transverse loading applied without shock.

(2) Impact bending test, which records its toughness or resistance to shock.

(3) Hardness test, which measures the resistance of the timber to indentation.

(In the first preliminary experiment only static bending tests were carried out.)

These tests showed that "brown oak" was inferior in strength to normal oak of the same density. The "brown oak" was equal in density to timber from the lower range of oak which had been tested previously. The wood of the "brown oak" was markedly softer and more brittle in comparison with normal oak of equal specific gravity. The decrease in strength ranged from 11 per cent in static bending at 12 per cent moisture content to 32 per cent in hardness when in the green condition. These results explain the fact that the wood of "brown oak" is milder and therefore easier to work than the wood of normal oak. There is no need to apprehend the continuation of decay after felling and drying, and "brown oak" can safely be used for decorative purposes where great strength is not required.

A full account of this side of the investigation, with figures obtained from the tests, has already been published (6), and it is hoped that later it may be possible to carry out further tests on material obtained from field inoculation experiments, in order to gain an insight into the rate of progression of attack in relation to alteration in mechanical strength and to correlate this with chemical alteration in the wood, as has been done in Sitka spruce attacked by *Trametes serialis* Fr. and in ash attacked by *Polyporus hispidus* (Bull) Fr. The second series of tests shows that although the strength is not greatly impaired in certain respects, there is a very definite softening of the wood and the timber becomes "brash". The absence of any appreciable loss in strength in the first series can be accounted for mainly because the action of the fungus is probably not rapid.

Estimations of tannin content show a decrease of tannin comparable with that found by Groom; but the brown material does not appear to be a tannin, as on extraction little or no alteration in the colour has been observed.

It has not yet been found possible to extract the brown material for analysis, as it is insoluble in all the commonly employed reagents. Further work is in progress with the object of elucidating the chemical action of the fungus, which appears to be somewhat unusual. The fungus, as is shown by mechanical tests, acts on the cell walls of the wood, but as its action is not rapid and does not cause much loss in strength in the early stages, it appears unlikely that the cellulose is acted upon to any great extent initially, and it is suggested that the action is probably on the tannin, on the lignin constituents of the wall and on the pectic substances (possibly the hemicelluloses), the

brown substance representing the residue. Such action probably would not cause much loss in mechanical strength, though it might soften the timber.

General discussion

In the tree from near Radley there was very little sign of decay although, judging from the extent of "brown oak" formation, the fungus must have been present for many years. Examination of the fungus in the wood shows that, apart from occasional bore holes, there is little sign of cell-wall destruction such as cross cracking or general thinning, as is usually found in wood attacked by an active wood-destroying fungus. These facts, in combination with the presence of the deep colouring matter in the cells, point to a somewhat unusual type of metabolism.

The two most common hosts of *Fistulina hepatica* are oak and sweet chestnut, both of which contain abundant tannin; the number of species of fungus which attack these two trees is limited, further suggesting a somewhat specialized metabolism. This may be an acquired toleration to tannin as is possibly so with forms which produce serious decay in oak, such as *Polyporus sulphureus*; or it may be, not so much that tannin acts as a toxic agent as that it reduces the amount of oxygen available and that such fungi require less oxygen. The fact that the dense timber of yew (*Taxus baccata*), is readily attacked by *Polyporus sulphureus*, supports this view. A third possibility is that some fungi may be able to obtain part of their food material by breaking down the tannin and obtaining sugars from it. This appears to be the most likely hypothesis for *Fistulina hepatica*, which is apparently able to obtain its food material from the heartwood for a long time without affecting the cell wall of its host. The biology of this fungus should afford an interesting study for the forest mycologist in co-operation with the biochemist.

The work of Dr R. C. Fisher, entomologist at the Forest Products Research Laboratory, shows that wood partially decayed by *Phellinus cryptarum* Karst. is preferred by the Death Watch beetle to sound wood. It is possible that "brown oak" may prove to be more liable to Death Watch attack, but so far no definite evidence on this point has been obtained.

Summary

1. *Fistulina hepatica* has been shown to be the cause of "brown oak", and of a similar discoloration in sweet chestnut (*Castanea sativa*).

2. The cultural characteristics of the fungus are described.

3. A description of the fungus in wood and of its behaviour in culture are given.

4. The optimum temperature for growth in culture was found to lie between 25 and 27° C.

5. Preliminary experiments on the metabolism of *Fistulina hepatica* indicate that it is in some respects different from that of a normal wood-destroying fungus causing a brown rot: the fungus may obtain a portion of its sugar supply from the tannin. It is suggested that the brown amorphous material may be a degradation product of the lignin and hemicellulose brought about by the action of the fungus.

6. Results of mechanical tests (6) show that no appreciable loss in strength is caused in the early stages by the action of *F. hepatica*, but later considerable softening takes place and the timber becomes somewhat brittle.

7. There is no risk of trouble being caused by the continuation of decay in the converted timber, and the fact that "brown oak" is easier to work than normal oak is an advantage where great strength is not required.

To W. A. Robertson, Esq., Director, Forest Products Research Laboratory, thanks are due for permission to publish this paper.

In connexion with the provision of material, assistance was kindly given by Radley College Estate and Dartington Hall, Ltd.

Grateful acknowledgement is also made to my colleague, W. G. Campbell, Esq., for assistance with the chemical work, and to W. R. Hutchins, Esq., for the photography.

EXPLANATION OF PLATES I–VII

Plate I

Fig. 1. Illustrations of brown (A) and normal (B) oak.
Fig. 2. Illustrations of brown (A) and normal (B) chestnut.

Plate II

Fig. 1. Photomicrograph of tangential longitudinal section showing reddish brown material in medullary rays and wood parenchyma. × 250.
Fig. 2. Ditto. Radial longitudinal section. × 375.

Plate III

Four-week-old culture of *Fistulina hepatica* isolated from "brown oak" growing on 5 per cent malt agar acidified with malic acid.

Plate IV

Fig. 1. Incipient sporophore formed at point of inoculation on 5 per cent malt-agar medium after 2½ months.
Fig. 2. Tongue-shaped sporophore developed on 5 per cent malt agar in the dark.
Fig. 3. Sweet chestnut showing brown coloration caused by *F. hepatica*.
Fig. 4. Photomicrograph of radial longitudinal section of wood of sweet chestnut cut across brown-coloured heart and showing hyphae of *F. hepatica* in the medullary-ray cells × 225.

A

B

Fig. 1

A

B

Fig. 2

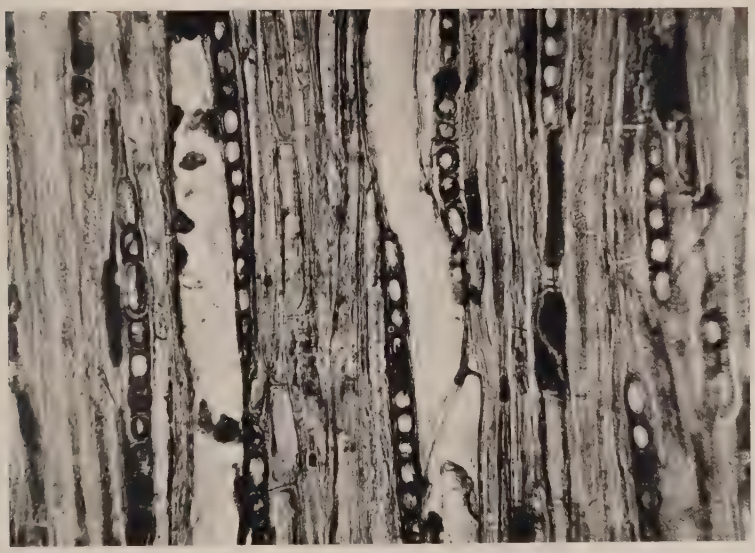


Fig. 1

Fig. 2

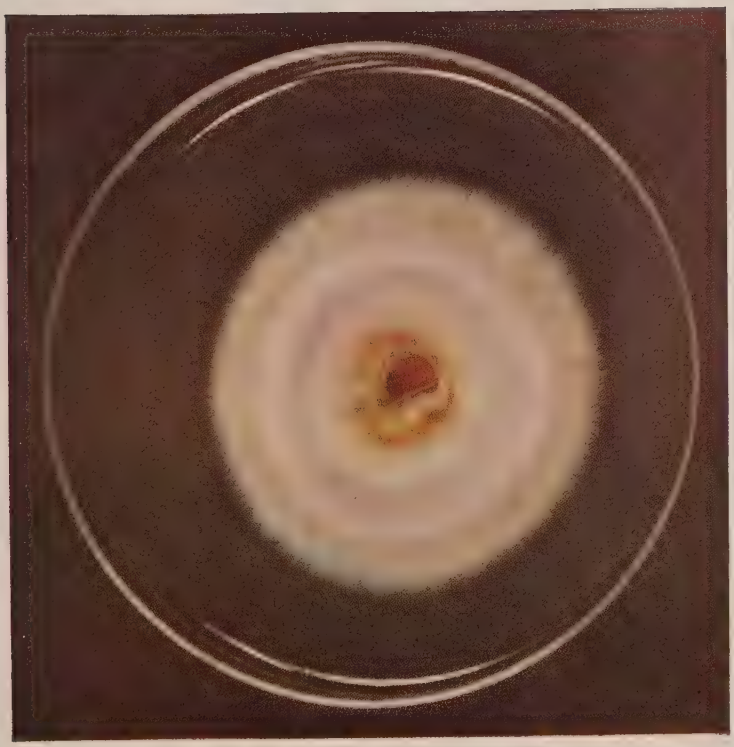


Fig. 1

Fig. 2

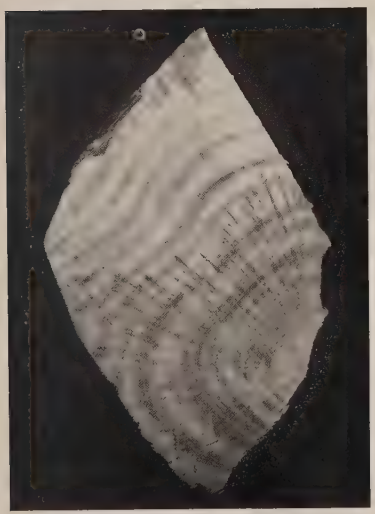


Fig. 3

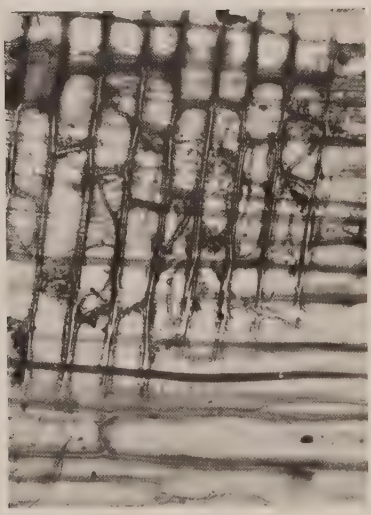


Fig. 4

Fig. 1

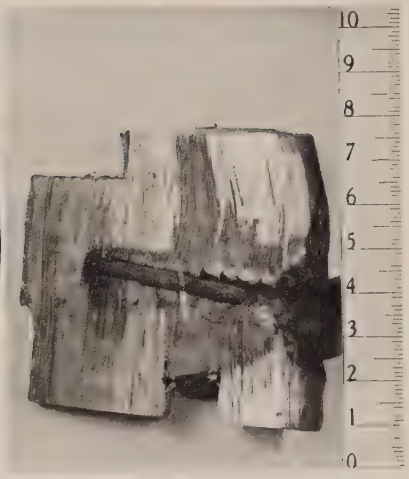


Fig. 2

Fig. 3

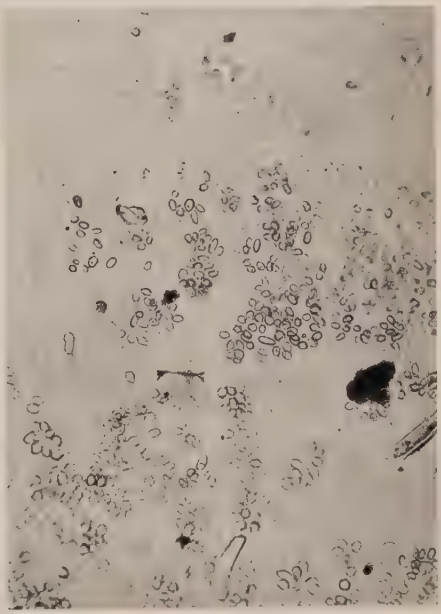
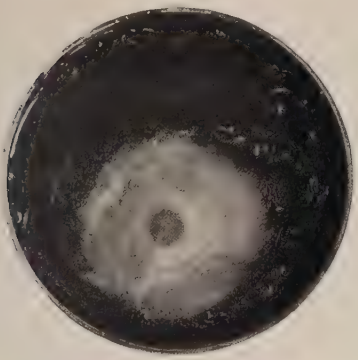
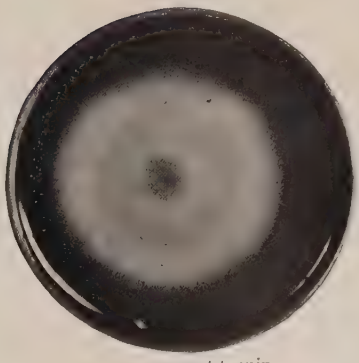


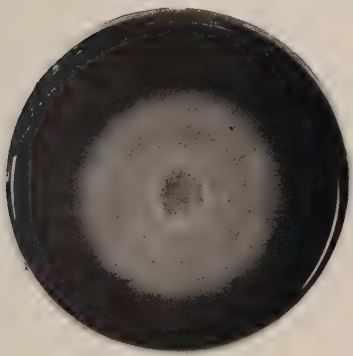
Fig. 4



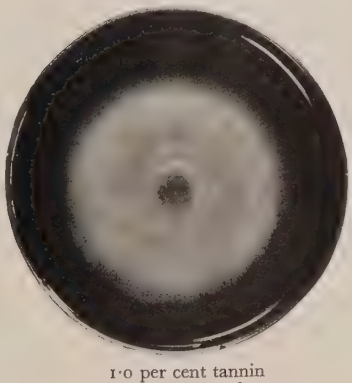
1·25 per cent tannin
2·00 per cent agar
(medium sloppy)



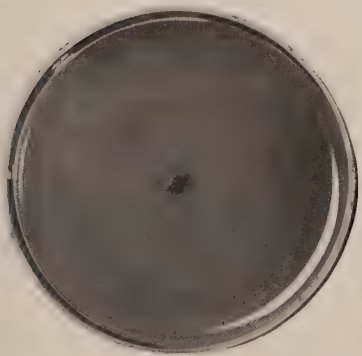
1.25 per cent tannin
5·00 per cent malt
2·00 per cent agar



1·0 per cent tannin
2·0 per cent agar



1·0 per cent tannin
5·0 per cent malt
2·0 per cent agar



Control
2 per cent agar

Control
5 per cent malt 2 per cent agar

PLATE V

Fig. 1. *Polystictus versicolor* showing abnormal growth after ten weeks on malt-agar medium to which 1 per cent tannin extract had been added.
Fig. 2. Portion of oak branch inoculated by means of Pressler increment borer. After seven months, showing discoloration caused by *F. hepatica*.
Fig. 3. Oak tree from Radley at commencement of felling operations showing brown heart.
Fig. 4. Photomicrograph showing secondary spores formed in culture. × 400.

PLATE VI

Top portion of plank from Radley oak tree showing distribution of the brown heart at the upper extent to which the infection has reached. Note that in this area the brown coloration follows the outer limit of the heartwood on the left but that on the right normal coloured heartwood is also present.

PLATE VII

Petri-dish cultures showing stimulation of growth by the addition of oak wood tannin extract.

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FURTHER OBSERVATIONS ON THE SYSTEMIC INFECTION OF *LOLIUM*

By KATHLEEN SAMPSON, M.Sc.

(With Plates VIII and IX and 20 Text-figures)

I. A second endophytic fungus in *Lolium perenne*

In a previous study of the endophytic fungus which frequently occurs in certain species of *Lolium*, pedigree cultures of *L. perenne* were kept under observation for a number of years. The microscopic examination of pith scraped from a flowering stem and stained with cotton blue offered a simple and efficient method of detecting infected individuals in a mixed population (16). One of the parents selected in 1929 at the inception of the experiment was noted as containing a mycelium which retained only a little of the blue stain. As the work progressed, this "faint" type of mycelium was recognized repeatedly and without difficulty and noted as such in the records. Subsequent results extending over a period of six years have shown that it may be transmitted either by seed or by vegetative propagation of the host in which it produces no obvious symptoms of disease. To this extent it resembles what may be termed conveniently the first *Lolium* fungus. It differs from this organism, however, in certain mycelial characteristics, in its failure to form a thick hyphal layer in the seed, and above all in the fact that it can readily be brought into culture on agar media. Unfortunately, apart from the production of microconidia, it has remained persistently sterile, and like the original *Lolium* endophyte it has still no certain taxonomic position. The differences between the two types of infection in *Lolium* and in the invading fungi themselves are, however, of such a nature that it seems at present more reasonable to regard them as distinct organisms than to suggest any direct relationship between them. Evidence for this view will appear in the description which follows. Both types are also considered to be distinct from *Epichloe typhina*, which causes a similar systemic and latent invasion of *Festuca rubra* (15).* It appears, therefore, that at least three fungi may lead a barren and seemingly

* Since the work on *Epichloe typhina* was published, further evidence of latent infection came accidentally to my notice. A plant of *Festuca rubra* which had been used successfully for a number of years by Dr Jenkin in his genetical work was found to carry mycelium which proved to be *Epichloe typhina*. The fungus caused no appreciable damage and never fruited in clonal lines of this plant.

innocuous existence in association with members of Gramineae. *Festuca* and *Lolium* are somewhat closely related but we have no evidence as yet that there is any near affinity between the invading fungi.

Transmission by seed and propagants

The experimental work has been carried out with pedigree cultures of plants originating in a single individual (No. 1972) selected in 1929. Since open pollination was permitted, the maternal parent alone could be recorded. Parallel with the progeny trials were various series of propagants obtained by the vegetative division in autumn of plants from which seed had been taken in the previous summer. The data collected over a period of six years are summarized in Table I. In column A appear the results from plants derived

Table I. *Pedigree populations of* Lolium perenne (*No.* 1972) *selected* 1929 *as containing "faint" mycelium*

		A. Parent infected. Mycelium in flowering stem		B. Parent fungus free. Mycelium in flowering stem	
Year		Present	Absent	Present	Absent
1930	Progeny	4	6	—	—
1931	Progeny	6	14	—	—
1932	Progeny	9	6	0	10
1933	Progeny	60	22	0	60
	Propagants	20	0	0	12
1934	Progeny	17	15	0	10
	Propagants	22	7	0	12
1935	Propagants	8	3	0	7

directly from parents which were known to be carriers of the "faint" type of mycelium. In column B are recorded the observations made upon the progeny and propagants of certain fungus-free plants which appeared from time to time in the cultures. The relatively large number of negative results recorded in the infected series (column A) is due to two reasons. In the first place, the faint mycelium was not so easy to trace in a pith-scraping as the deeply stained mycelium of the first endophyte (cf. (16), Table III, p. 341) and was undoubtedly overlooked sometimes. Such errors were detected by the examination of additional stems from particular plants and by observations on propagants in a subsequent season. The same procedure proved that negative results were sometimes due to the failure of the fungus to penetrate every part of a plant and the consequent appearance of fungus-free individuals in the pedigree lines. When such plants were multiplied by seed and by division they remained non-infected during an experimental period of four years (Table I, column B). Incidentally it may be noted that parallel cultures of *L. perenne* (No. 1975) derived from a plant selected as fungus-free in 1929 also remained true for

this character during the complete period and the same holds good for the lines of *L. perenne* (No. 1971) which escaped infection by the first endophyte ((16), p. 341). The origin of fungus-free races was rarer in the latter type of infection than it was in the second endophyte now under discussion. There is no reason to think that either type of infection came from the soil.

Although transmission of the present fungus by seed does not proceed without exception, a high proportion (over 70 per cent) of infected individuals does occur in the families which come directly from an infected female plant. Vegetative propagation is evidently even more efficient as a means of disseminating the fungus, since transmission occurred with one possible exception in all the propagants taken from infected plants.

During the period of this experiment, which covers five generations of the host, the characteristics of the mycelium and the type of infection have shown no sign of change. Fructification of the fungus on the host has been sought for in vain.

In view of the comparative ease with which fungus-free races can be obtained in cultures of rye-grass containing this particular endophyte, it might seem a simple matter to estimate the effect of the fungus upon the general growth of the plant. That this cannot easily be done arises from the fact that *L. perenne* is a cross-pollinated species and therefore markedly variable in both vegetative and floral characters. All that can be said is that the presence of the fungus is not associated with necrosis of tissue or with any visible pathological symptoms. Possibly more exact information concerning its effect on the host may be forthcoming from a study of the growth of reciprocal crosses between infected and non-infected plants.

Characteristics of the mycelium and its distribution in the plant

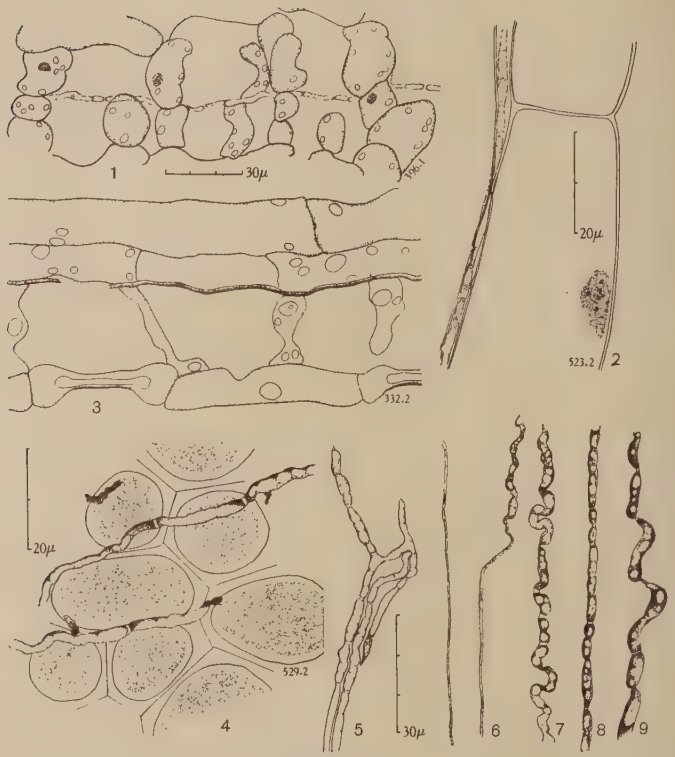
Mycelium is usually present in abundance in the pith scraped from the stem of an infected plant carrying the second endophyte of *Lolium* but it absorbs comparatively little of a protoplasmic stain, making a "faint" preparation in contrast to the richly coloured mycelium in a stained mount of the first endophyte. The differences seem to rest chiefly on the fact that the cells of the former have large vacuoles and sparse cytoplasm, while the latter is only slightly vacuolate and the contents of the cells look dense. This contrast is so marked that the two types can be distinguished with a high degree of accuracy (Pl. VIII, figs. 1, 3, and Text-figs. 5, 8, 9). Both fungi have rather closely septate mycelium but in the second endophyte the branching is more frequent and parallel strands or even wefts of mycelium are more often seen in pith preparations. The mycelium of the original endophyte, whether it is in *L. perenne*, *L. temulentum* or

L. remotum, usually occurs in the pith as long separate strands, often so much constricted at the numerous septa that a chain-like effect is produced. This is enhanced by a slight contortion which appears to result from elongation occurring in a limited space. A similar effect is sometimes produced in the mycelium of *Epichloe typhina*, but normally this fungus is characterized by the exceedingly fine straight lengths of mycelium which can usually be distinguished in pith mounts from both the fungi of *Lolium* (Pl. VIII, fig. 2 and Text-figs. 6, 7). In microtomed sections of leaves, mycelia of *Epichloe typhina* and the first *Lolium* endophyte look almost exactly similar.

Experimental evidence for the transmission of the second endophyte by seed and by vegetative propagation led to a search for the fungus in different parts of the plant. Portions of root, tiller buds, stems, ovules and ripe caryopses were fixed, embedded and stained with gentian violet by the methods previously used with *Epichloe typhina* (15). For comparison, material of *Lolium perenne* containing the true endophyte was likewise fixed and studied.

Mycelium of both the fungi of *Lolium* is intercellular. The second type, which absorbs little stain, is not nearly so easy to trace in the different tissues as the first, but both have been found widely distributed in the tiller buds of infected plants in both summer and winter. Text-figs 1, 3 show sections of young leaves from two infected plants with the characteristic mycelium of their particular endophytes crossing the intercellular spaces of the spongy parenchyma. I have not found either fungus in the root, but the difficulties in detecting it there are considerable and consequently little reliance can be placed on a negative result.

Passage up the flowering stem has been mentioned above. The pith in particular carries many parallel strands of mycelium. Invasion of the ovule and seed by the true endophyte has been described by other authors (5, 7). The most characteristic feature of this fungus seems to be the thick zone of mycelium which it forms in the ripe caryopsis outside the aleurone layer (Pl. IX, fig. 7). Such a zone has never been found in fruits containing the second endophyte. There is no doubt, however, that invasion of the flower occurs. Mycelium of the "faint" type has been seen in sections of the lodicules, at the base of the young ovary and in several mature fruits. In Pl. VIII, fig. 6 and Text-fig. 4 it is shown lying over some aleurone cells which are seen in surface view. In the ripe fruit it has been found only in this position, never in the embryo, but this may be due to the difficulty in detecting it among closely packed cells. This difficulty makes it impossible to decide by microscopic methods how many fruits do or do not carry this fungus. Data from the pedigree cultures indicate that while examples of failure are certainly not rare, seed transmission occurs in a high percentage of cases.



Text-figs. 1–9

1. Leaf of *Lolium perenne* (No. 1972) collected in February 1933 and cut in longitudinal section, showing the faintly stained mycelium of the second endophyte traversing the spongy parenchyma.
2. Mycelium of the same fungus in the leaf sheath of a young shoot collected in July. The mycelium is highly vacuolate like that taken from pith (Fig. 5).
3. Leaf of *Lolium perenne* (No. 1971) in longitudinal section showing the deeply stained mycelium of the first endophyte. It is scarcely distinguishable from that of *Epichloe typhina* in leaves of various grasses (15, Pl. VIII, fig. 1).
4. Mycelium of the second endophyte lying over some aleurone cells in a ripe caryopsis of *Lolium perenne*.
5–9. Mycelia from pith scrapings stained with cotton blue. 5. Branched vacuolate mycelium of the second endophyte of *Lolium perenne*. 6, 7. *Epichloe typhina* from a healthy inflorescence of *Dactylis glomerata*, showing the typical slender form grading into a coarser type which more nearly resembles that of the *Lolium* endophyte (8, 9).

Isolation and growth in culture of the second endophyte

It is not difficult to remove long strips of pith from grass stems and to place them on solidified agar without serious contamination. Flowering stems from plants carrying *Epichloe typhina* treated in this way yielded pure cultures of that fungus(15). In May 1933 experiments were set up to see if this technique might provide a method of bringing into culture either of the *Lolium* fungi. Immediate success was obtained with the second endophyte. Strips of pith laid in close contact with clear nutrient agar were examined microscopically at intervals and the mycelial strands present were seen to branch, putting out rather fine, straight hyphae which finally formed a silky-looking colony. It was not unusual to obtain a pure culture immediately, all the pieces of pith on a plate giving the same type of mycelial growth. Contaminations which occurred on some plates were easy to detect.

In 1933, twenty-seven separate isolations, taken from three distinct plants, were kept in culture for a time but they showed no noteworthy differences. Fresh isolations made in 1934 and in 1935 agreed with stock cultures from the first isolation experiments. Since all the material originated from one plant, it is probable that all isolants represent one strain of the fungus.

Results of inoculation experiments are not available,* but there is little doubt that the cultures represent the "faint" type of mycelium in the pith, since the initial stages of growth of this mycelium have been traced under the microscope and the same fungus has been isolated many times over a period of three years. Moreover, plants lacking the fungus were tested occasionally by placing strips of their pith on agar but this remained free from mycelial growth.

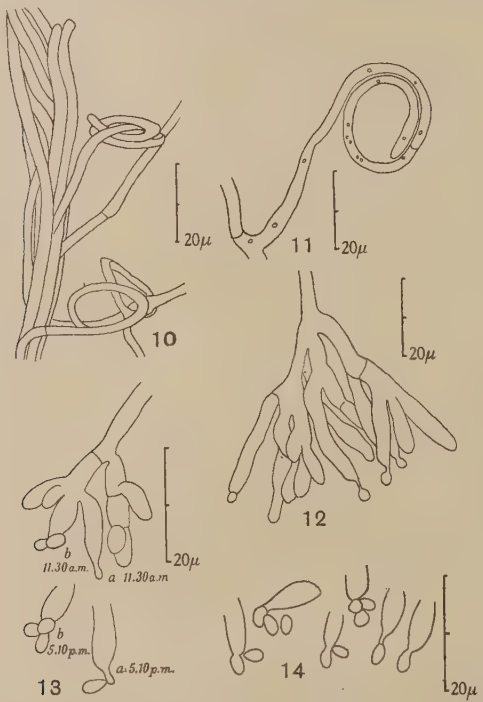
The fungus grows readily but somewhat slowly on a wide range of substrata, producing on agar media rather compact fluffy aerial mycelium, white to cream colour, in appearance like a miniature powder puff, while the submerged mycelium is often accompanied by a chrome-yellow stain, especially striking on oatmeal agar. Under a lens the aerial mycelium is seen to be funiculose, especially when lying near glass.

In branching and vacuolation the mycelium from cultures is not unlike that taken direct from the pith of the plant. In addition it produces rather numerous spirals (Text-figs. 10, 11), especially when grown upon natural media such as potato, rice and wheat grains. Spirally coiled hyphae of a somewhat similar form have been seen in *Ctenomyces* spp. and certain other Dermatophytes(9, 14).† The natural media mentioned also provide good crops of microconidia,

* Since this paper was written definitely positive results have been obtained from inoculation experiments with the second endophyte.

† I am indebted to Dr E. J. Butler and to Mr E. W. Mason of the Imperial Mycological Institute for these references.

though these were seen sometimes on the growth arising directly from the pith lying on agar. The microconidia arise in succession from the clavate tips of closely branched, penicillate conidiophores (Pl. VIII, fig. 4). The type of branching and the method of budding off of conidia is shown in Text-figs. 12–14. The conidia, which are not



Text-figs. 10–14. The second *Lolium* endophyte in culture

10. Rope of mycelium with spiral coils.
11. Origin of a spiral; material taken from a culture on potato.
12. Microconidiophora somewhat simplified.
13. Young conidiophore formed on a hanging drop of malt agar showing the abstriction of microconidia.
14. Sterigmata and microconidia.

formed very abundantly, seldom or never remain in chains but tend to adhere together and to form with the conidiophores minute hyaline masses which become waxy-looking in dry cultures. The conidiophores usually arise from long strands of mycelium but occasionally they are formed in numbers from the cells of one of the coils (Pl. VIII, fig. 5). The conidia are hyaline, oval, and measure

2–2·5 × 1·5 μ. They resemble those associated with species of *Sclerotinia* (18) and *Botrytis* (3), but there has been no sign of sclerotial formation in any culture of the *Lolium* fungus.

II. The growth of the first endophyte in culture

All previous attempts to isolate the *Lolium* fungus have been made either with the fruit of the grass (usually *L. temulentum*) or with young seedlings; no two authors have obtained the same organism. The isolations which have been regarded as representing the endophyte are a smut (11), *Fusarium* sp. (8), *Alternaria* sp. (1) and more recently *Chaetomium* sp. (10).

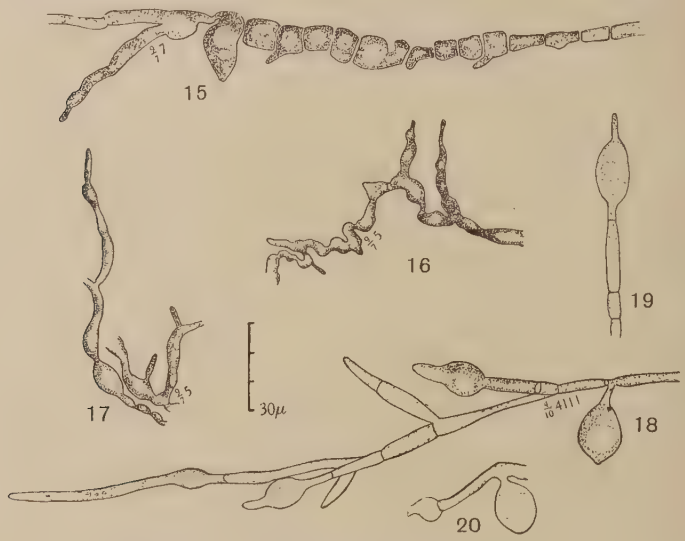
Efficient sterilization of seeds is known to be difficult. Following the technique of Guennewig (10), grains of darnel (No. 547) carrying the endophyte were treated with a solution of bleaching powder, washed and put to germinate under aseptic conditions. Later seedlings were transferred to a nutrient solution in small flasks. Some became coated with mycelium, others remained superficially sterile and elongation of the shoot continued for some days. After a week the coleoptiles of the "sterile" seedlings were fixed and sectioned and the typical endophyte was found therein. Sterilization had clearly not affected the growth of the fungus in the plant, but it showed no signs of leaving the host tissues and growing out into the medium. This suggests that the various moulds present on some seedlings represent fungi on or within the pericarp which the sterilizing agent failed to destroy.

The presence of abundant mycelium in pith suggested that the fungus might be brought into culture by the technique which was successful with *Epichloe typhina* and the second endophyte of *Lolium perenne*. In the first trials, started in 1933, strips of pith from *L. perenne* (No. 1971) taken with every possible precaution against contamination, were placed upon the surface of an agar plate and examined at intervals under the microscope. Although the mycelium of the endophyte was clearly visible in the pith, no growth from the latter occurred. Stained preparations of strips of pith which had rested on nutrient agar for over a week showed that the hyphae had swollen and put out very short branches, but these made no further growth (Text-fig. 15). In contrast to this, pith containing *Epichloe typhina* and the second *Lolium* endophyte readily yielded their characteristic growth (p. 89).

In 1934 further tests were carried out. In collaboration with Mr C. G. C. Chesters, attempts were made to grow the fungus by placing strips of pith from infected plants of *L. perenne* and *L. temulentum* in media containing the expressed juice of darnel stems which was rendered germ-free by passage through a Seitz Werke No. 6

filter.* These experiments again gave negative results, but Mr Chesters made the valuable suggestion of trying an egg medium which Ayers found successful in cultivating *Dispira cornuta* (2).

The medium was prepared by mixing the white and yolk of a fresh egg lightly together, coagulating it by steam in a cylindrical vessel 1½ in. in diameter, cutting slices of the "scrambled" egg column about ½ in. thick and placing these disks in small Petri dishes. Sterilization was affected in the autoclave and the lid of each dish



Text-figs. 15–20. First endophyte of *Lolium perenne* (1971)

15. Mycelium in pith after nine days on prune agar.
16, 17. Mycelium in pith after three days on egg medium.
17. Branches with intercalary swellings.
18–20. Mycelium from sub-culture on potato-dextrose agar with intercalary swellings.

was only removed to allow one or two strips of pith to be placed on the smooth, slightly honeycombed surface of the medium.

The first experiment was started on 4 July. On the fifth day slightly opaque lines could be seen in the pith when examined under a lens. Pieces stained with cotton blue showed that growth had occurred in the long parallel strands of mycelium. Growth, which was visible to the naked eye after eight days, was at first restricted

* This section of the work was carried out in the Department of Botany of the Birmingham University in order that use might be made of the efficient press for sap extraction designed by Dr W. Leach (12). I am indebted to Prof. W. Stiles or the kind hospitality of his laboratory.

to the pith. It consisted of more or less spherical cushions which finally joined together to form a raised mass about 2 mm. in diameter, varying in length with the size of the strip of pith. Superficially the growth resembled a bacterial colony but it was firm to the touch and under a lens the surface was seen to be covered with short, erect, aerial hyphae. The growth was hyaline or cream coloured, not offering much contrast to the egg itself. In this initial experiment, each of six dishes gave identical results and none was spoilt by contaminations.

A more extensive experiment was at once set up which included malt and prune agar as well as egg medium, and pith was taken from *Lolium perenne* (No. 1971) carrying the first endophyte, *L. perenne* (No. 1972), carrying the second endophyte and infected plants of *L. temulentum* (No. 547). Pith from the first-named plant gave the same type of growth on egg but no macroscopic growth on the two kinds of agar. *L. perenne* (No. 1972) yielded cultures of the second endophyte on both egg and agar though it did not flourish on the egg medium. *L. temulentum* failed on all the media.

Further repetitions produced the same results and by staining samples of pith at intervals various stages in the growth of the mycelial strands were observed under the microscope (Pl. IX, figs. 10, 11, and Text-figs. 16, 17). Branches frequently showed the intercalary swellings which were seen in subsequent subcultures.

Transfers of the growth from *L. perenne* (No. 1971) on egg were made to fresh dishes of the same medium and some of these were left in a sterilized moist chamber during the vacation. These remained free from contamination and the cultures were multiplied in October by fresh transfers to egg.

In November an attempt was made to transfer to different media. When pieces of the growth were first placed on the surface of agar, lysis rapidly occurred in many cells but when the inoculum was rather large, some pieces of the mycelium survived and after a few weeks produced a glabrous convoluted growth on the surface of nutrient agar. When this was taken back to egg it made a colony similar to that which developed from the original pith. Transfers from egg to agar media and vice versa have been made many times with the same results. Some lysis always occurs, but most of the transfers are successful in establishing a subculture. The fungus has now been grown on a variety of media including potato disks and agar prepared with Knop's solution, Lemco, egg, liver extract, malt, potato dextrose, oatmeal and on gelatine containing egg. Growth on the last medium was sparse and little raised from the surface. Gelatine was not liquefied. On all the agar media growth was raised, forming smooth brain-like folds of about the same colour as the agar. Aerial hyphae were absent (Pl. IX, fig. 12).

Experiments in 1935 were intended to re-confirm the differential effect of egg and agar in starting initial growth, and to extend the work to other species of *Lolium*.

The first set of cultures was made on 29 May with *L. perenne* (No. 1971). Each of four dishes containing egg to which pith was added gave the same type of growth as subcultures from isolations made in 1934. No growth developed from pith placed on malt agar, and no growth was obtained on egg or malt when the pith was taken from *L. temulentum* (No. 547).

During June and July this experiment was repeated a number of times using young and old stems, and the following facts were established:

(1) The endophyte from *L. perenne* (No. 1971) was successfully isolated only when egg was used to stimulate the initial stages. Afterwards the fungus would grow on agar media provided the inoculum was sufficiently large.

(2) Negative results with pith containing abundant mycelium were always obtained with *L. temulentum*, *L. temulentum* var. *arvense* and *L. remotum*, while *L. perenne* (No. 1971) invariably yielded typical colonies on egg. There is perhaps an indication here of strain differences in the endophytic fungus of *Lolium*.

The microscopic features of the fungus in culture offer few points of interest. The growth consists of fine septate mycelia with a tendency to form rather frequent intercalary swellings (Text-figs. 18–20). Similar swellings appeared in the branches put out by mycelium in pith after three days' contact with egg (Text-fig. 17). It is unfortunate that the growth of this fungus as a saprophyte has so far not helped to establish its identity.

Discussion

I. It is evident that little can be said concerning the taxonomic position of the fungus described in this paper as the second endophyte of *Lolium*. It may, however, be useful to summarize the evidence for regarding it as distinct from other known species found in association with *Lolium* and related genera of Gramineae.

Epichloe typhina which causes a closely similar type of systemic infection in *Festuca rubra* readily produces its typical conidia in cultures obtained from infected pith, and forms neither the mycelial coils nor the microconidia characteristic of the present fungus. The same holds true for *Claviceps purpurea* which was isolated from sclerotia for purposes of comparison. Mycelial characteristics in the plant and in culture, coupled with the presence of microconidia, do not suggest any relationship with the smut fungi. These features and the ease with which the fungus adopts a saprophytic existence readily

distinguish it from that which we have called the first or original *Lolium* endophyte. When these two endophytes are closely compared they seem to have no features in common except sterility and habitat.

Recent evidence that the microconidia of certain Ascomycetes may function as male gametes (4, 5, 6), and the mass of data relating to heterothallism, raise the question whether the second fungus in *Lolium* may not be a unisexual form which needs a strain of the opposite sex in order to fructify. If this be so, identification awaits the chance discovery of the right strain and no changes in the nutrient conditions of cultures now available are likely to produce the desired result. On the other hand it may be a mutant which is permanently sterile. Until new facts are discovered it seems best to use a non-committal name such as that given in the title of this paper.

The growth of the first endophyte in culture

II. The cultural work with the first *Lolium* endophyte has not thrown any new light upon the identity of this organism except in a negative sense, since it has made it rather more difficult to accept the names already suggested. The possibility remains however that closely similar types of systemic infection may be caused by quite distinct organisms.

A fresh investigation of the *Lolium* endophyte was originally undertaken in the hope of finding a link between this organism and *Epichloe typhina*, but the culture work has brought to light points of difference rather than marks of similarity and the view that they might be identical must, I think, be abandoned.

It is interesting that another organism which appeared to be an obligate parasite has been brought into culture by the use of a highly concentrated protein medium and that after living for a time as a saprophyte it has grown almost equally well on various agar media. This behaviour and the fact that it is important to transfer large pieces of inoculum coincide with the results Sawyer obtained working with some entomogenous members of the Entomophthoraceae (17).

A further point of interest is the fact that so far only the strain living on *Lolium perenne* has been brought into culture. The endophytic mycelia in *L. remotum*, *L. temulentum* and *L. temulentum arvense* have not yet been cultured away from the host. It seems likely that a more extensive search for strains of both the *Lolium* endophytes might yield interesting results.

It is, of course, very desirable that those endophytes which have been brought into culture shall be successfully reintroduced into the host. At present, belief that cultures represent the fungi inhabiting these particular grasses rests upon the direct observations of the growth of the mycelial strands in pith strips and upon the consistent

development of the same type of growth in culture. Inoculation has not yet met with success.*

Summary

A description is given of the systemic infection of *Lolium perenne* by a fungus which does not fructify in the plant and which causes no pathological symptoms. It is transmitted by vegetative propagation and by seed. Mycelial characteristics, the fact that it does not form a thick zone of mycelium in the fruit and that it will grow readily as a saprophyte on all kinds of media distinguish it from the well-known endophyte of *Lolium*.

In culture, spiral coils and microconidia are formed. Since no other types of spore and no sclerotia have been seen, identification is impossible. For the present the organism is referred to as the second endophytic fungus of *L. perenne*.

Various attempts have been made to cultivate the first endophyte of *Lolium*. Using egg as a medium, cultures have been obtained which are believed to represent the fungus in *L. perenne* (No. 1971). After initial isolation on egg the fungus will grow in agar media but it remains sterile on all media tested and its culture has provided no clue to its identity.

Acknowledgements

I am indebted to Mr E. L. Hughes, M.Sc., Mr P. T. Thomas, B.Sc. and to Mr J. T. Jones, B.Sc., who gave valuable help as student assistants during several summer vacations, and to Mr J. W. Watkins for his care of experimental plants.

I wish to thank Prof. R. G. Stapledon, C.B.E., M.A., for making available the facilities of the Welsh Plant Breeding Station.

EXPLANATION OF PLATES VIII, IX

For assistance in photography I am indebted to Mr H. G. Osterstock, John Innes Horticultural Institute (Figs. 1, 2, 3, 7 and 8), to Mr J. H. Western (Figs. 5, 6, 10–12) and to Mr P. T. Thomas (Figs. 4 and 9).

Plate VIII

Figs. 1–3. Mycelia in pith-scrapings stained with cotton blue.
Fig. 1. First endophyte from *L. perenne* (No. 1971).
Fig. 2. *Epichloe typhina* from healthy panicle of *Dactylis glomerata*.
Fig. 3. Second endophyte from *L. perenne* (No. 1972).
Figs. 4, 5. Microconidia from culture of second endophyte. In Fig. 5 the conidiophores have developed from a spiral coil.
Fig. 6. Mycelium of second endophyte lying over aleurone layer in ripe caryopsis of *L. perenne* (No. 1972).

* See footnote, p. 89.

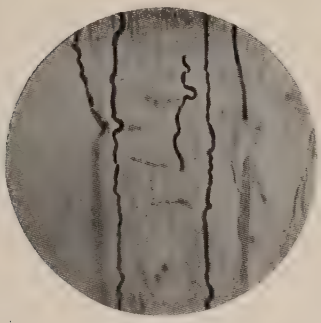


Fig. 1

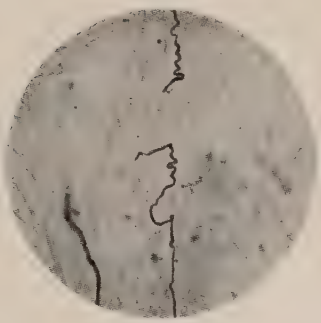


Fig. 2

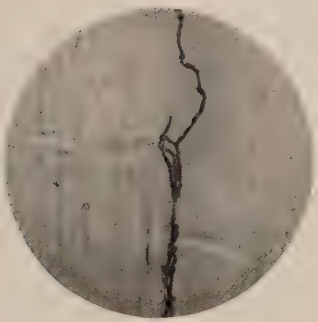


Fig. 3

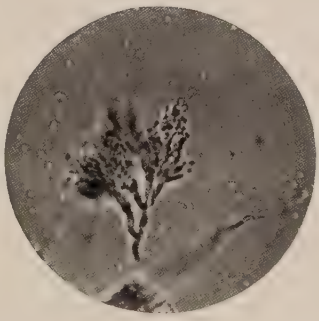


Fig. 4

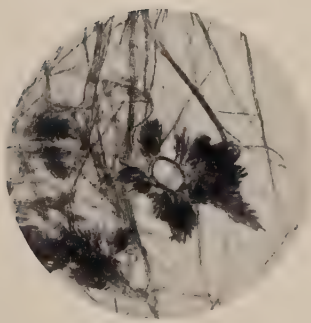


Fig. 5

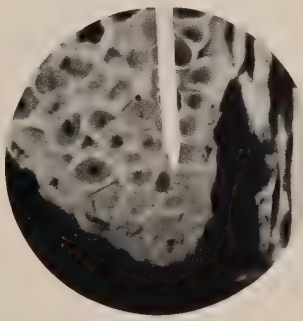


Fig. 6

Fig. 7

Fig. 8

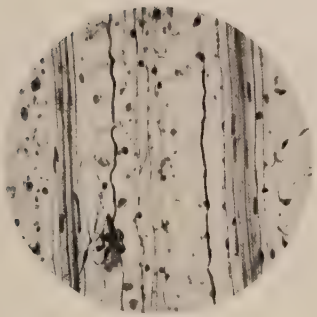


Fig. 9

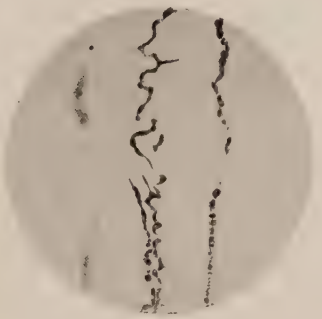


Fig. 10

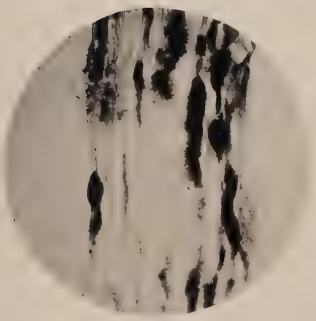


Fig. 11

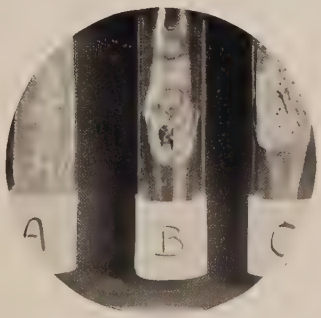


Fig. 12

PLATE IX. First *Lolium* endophyte

Figs. 7, 8. From ripe caryopses of *L. temulentum* cut transversely and stained with cotton blue.
Fig. 7. Infected female plant (No. 547) × non-infected male (No. 553).
Fig. 8. Reciprocal cross with mycelial layer absent.
Figs. 9–12. First endophyte from *L. perenne* (No. 1971).
Fig. 9. Mycelium perennating in tiller bud.
Fig. 10. Initial growth of mycelium in pith which has rested on egg for 3 days.
Fig. 11. Growth on same medium after 9 days.
Fig. 12. Growth on agar. A, potato-dextrose; B, malt; and C, ground oats.

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THE SPIRAL HYPHAE OF TRICHOPHYTON*

By A. M. DAVIDSON and P. H. GREGORY

(With 15 Text-figures)

The occurrence in cultures of Dermatophytes of helically coiled hyphae is a characteristic of Sabouraud's *microides* division of the genus *Trichophyton*. These spiral hyphae are not found while the fungus is living parasitically on the skin of the human or animal host, but are produced in great numbers when certain species of *Trichophyton* are grown in culture on artificial media. They are therefore organs which belong to the saprophytic phase of the Dermatophytes.

Matruchot & Dassonville (1899) drew attention to the resemblance between the spirals of the dermatophytes and the spiral ornaments, so-called, which surround the perithecia in *Ctenomyces serratus* Eidam. This resemblance was used by Langeron & Milochevitch (1930) to justify the inclusion of the Dermatophytes in the family Gymnoascaceae, although asci or functional perithecia are usually absent, and not known with certainty.

Little attention has been paid to these remarkable structures, though Grigoraki (1927) made their presence the basis of his genus *Spiralia*, in which he separated off the members of Sabouraud's group *microides*. The validity of this distinction was shaken when Langeron & Milochevitch (1930) showed that on media such as grains of oats *Microsporum felineum* could be induced to produce spirals which were up to that time unknown in that species. Emmons (1934) occasionally found spirals in cultures of the same species even on Sabouraud's maltose-peptone agar (*milieu d'épreuve*).

Spirals have been regarded usually as ornaments or curiosities of no particular significance. Thus Emmons says "like some other structures produced by the fungi, they are not believed to possess any particular function or evolutionary significance". The investigation here described was prompted by the idea that organs formed in such regularity and abundance as the spirals of *Trichophyton gypseum* are unlikely to be produced by such plastic organisms as the fungi without some significance in the life history. The long supposed functionless pycniospores of *Puccinia* may be cited as an example of the fallacy of regarding constantly occurring fungus organs as rudimentary. The

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development and structure of the spirals were studied, and as a result of experiments on the properties of mature spirals a tentative suggestion as to their function is put forward.

Although no Dermatophyte has yet been observed growing as a natural saprophyte, evidence is accumulating to indicate that under suitable conditions they can enter upon a saprophytic phase of growth. Recent investigations along this line have been reviewed by Gregory (1935). At present the saprophytic phase is known only under laboratory conditions. A study of spiral structure may therefore be expected to throw light on the nature of the saprophytic phase of these fungi.

Development of spirals

The stages in the development of spirals on a colony may be illustrated by the following description of a culture on wheat-flour agar. The culture was a typical strain of *Trichophyton gypseum* which had been derived from a single conidium (aleuriospore) isolated by the wet needle method of Hanna (1928). As noted by previous writers, these monoconidial cultures developed conidia (aleuriospores), macroconidia (fuseaux), and spirals as freely as cultures derived from mass inocula (Spring, 1931; Emmons, 1931). The medium was prepared by adding agar to a paste containing 1 per cent whole-wheat flour in water, and heating on a water-bath for several hours to remove air before sterilizing in conical flasks in the autoclave. Removal of the air was necessary to prevent frothing of the medium in the small containers.

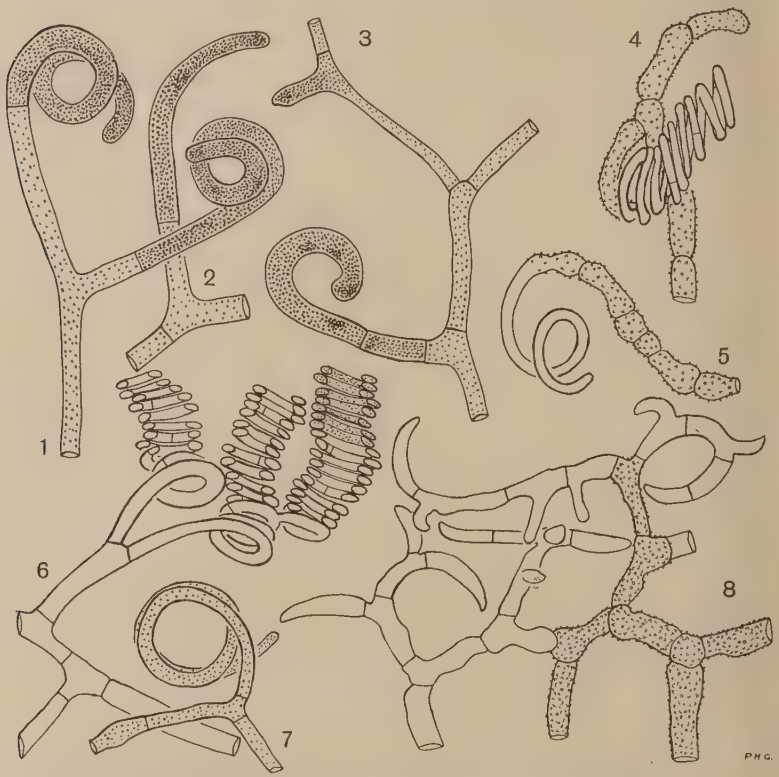
The cultures were kept at room temperature for four weeks, and at the end of that time had a diameter of 6 cm., corresponding to a radial growth of 7–8 mm. per week. The cultures had a chalky granular surface which showed pronounced zonation. From each zone portions of mycelium were removed and examined microscopically. The occurrence of spirals in the various zones is shown in Table I.

Table I. *Occurrence of spirals in various zones of culture of* Trichophyton gypseum

Zones	Condition of spirals
(1) Outer translucent zone of radially growing pioneering hyphae immersed in medium, forming edge of colony	No spirals
(2) Elevated white downy zone free from spores	No spirals
(3) White powdery zone about 1 cm. in breadth containing numerous conidia (aleuriospores)	Spirals developing as in Figs. 1–3
(4) Cream-coloured powdery zone occupying greater part of colony	Mature (empty) spirals abundant
(5) Downy central tuft over inoculum	No spirals formed

From examination of different parts of the third zone it was concluded that conidia are produced somewhat earlier than spirals, since

conidia were found nearer the edge of the colony than were spiral initials. The zone of active spiral production was estimated to be from seven to ten days old.



Figs. 1–6 and 8. Development and structure of spirals of *Trichophyton gypseum*.
Figs. 1–3. Stages in development of spiral and establishment of asymmetry. Culture on 1 per cent wheat agar. (× 1100.)
Figs. 4, 5. Mature spirals borne on torulose hyphae. Culture on wheat agar. (× 485.)
Fig. 6. Well-developed branched spirals from *in situ* culture on hair naturally infected by *T. gypseum*, kept in a saturated atmosphere. The distal portions of the spirals represented in section to show flattening of component hypha in a plane at right angles to the spiral axis. (× 1100.)
Fig. 7. An aberrant spiral of *T. interdigitale* with clockwise asymmetry. (× 485.)
Fig. 8. Crooked hyphae often associated with spirals on mycelium. (× 1100.)

The spirals begin either as short lateral branches or as terminal cells on aerial hyphae which have somewhat denser protoplasm than the ordinary hyphae. Most commonly the spirals arise in a lateral

position on an aerial hypha. One of the cells of the hypha develops a growing region, and puts out a lateral diverticulum at right angles to itself; this arises somewhere between the middle of the cell and the distal septum which separates the cell from the younger cell in front. A lateral hyphal tip is thus established and growth continues. The new hypha is not as a rule cut off from the parent cell by a septum at its base, but by a septum laid down a short distance along the hypha. The basal cell of the branch thus becomes T-shaped. When the second or third septum has been formed the tip of the hypha begins to curve to one side, and this curvature is continued in all successive cells of the spiral. Continued growth around an arc would soon bring the tip of the hypha into contact with the older portion of the same hypha. However, before this occurs a lateral displacement of the growing tip becomes evident, and the tip passes, in the course of its growth, to one side of the previously formed part of the curved hypha. An asymmetrical structure is thus produced (Fig. 1). As will be shown later, it is evident from observations on mature spirals that this lateral displacement of the growing tip is always to one particular side of the older hypha.

The developing spiral becomes cut off from the parent hypha by a septum, and as the spiral grows at the tip its protoplasm moves forward, leaving the hypha empty behind. Stages were found in fixed preparations in which the older parts of the structure were empty, only the cell walls being visible, while the growing tip was full of refractive protoplasm which stained with methylene blue (Fig. 6). Direct observations on living spiral hyphae showed that they made from a half to one complete turn in twenty-four hours.

Structure of mature spirals

Fully mature spirals are empty tubes; they fail to take up cytoplasmic stains. Presumably the protoplasm has been exhausted in the laying down of the spirally coiled wall. In one experiment two mature spirals were removed from the mycelium with needles, and placed on Sabouraud's agar. They were observed at intervals under the microscope for several days, but as they showed no signs of germination, they are regarded as dead structures. The emptying of the older part of the hypha is common in the Dermatophytes as in many other fungi. Sabouraud (1910) noted that the protoplasm in sporogenous hyphae became encysted in the spores, leaving the conidiophore empty. There is a striking difference between an emptied spiral and an emptied conidiophore. A conidiophore, or an ordinary vegetative hypha, when emptied of its protoplasm is a shrunken irregular structure which tends to collapse. When mounted in a refractive medium such as glycerine jelly it is almost invisible. The emptied portion of the spiral,

on the other hand, is a smooth, strong, easily visible tube, showing no tendency to collapse or become irregular in outline. The wall of the spiral is apparently thicker and more durable than that of the conidiophore. Spiral hyphae have not so far been observed to bear spores, though they usually arise in close proximity to spore-bearing hyphae. They are always aerial. The hypha which bears the spiral may be stout and torulose (Fig. 4). Sometimes in place of spirals these torulose hyphae bear peculiar crooked hyphae (Fig. 8) of unknown significance.

When mature and empty, the spirally coiled hypha is flattened from side to side. The cross-section of the component hypha is an ellipse whose major axis is at right angles to the axis of the spiral. This flattening can be seen by focusing upon a mature spiral which may be either in air or mounted in glycerine jelly. When the objective is focused on the plane of the axis of the spiral so that the component hypha is seen in optical section, it is apparent that the wall of the hypha is flattened above and below as shown in Fig. 6. Whether the flattening arises by mutual pressure of the coils of the spiral during growth or as a result of unequal growth of the cell wall is not known. The flattening probably has a mechanical significance which will be dealt with when the possible function of the spirals is considered below.

In the older parts of a culture, spirals of from one to thirty turns can be found. The greater number of turns has been found in specimens prepared by the *in situ* culture technique (Davidson & Gregory, 1934).

When the mycelia are growing under favourable conditions some of the spirals may be branched, a new growing tip being established at some point on the spiral curve. Growth is at first radially away from the parent spiral, but soon the tip of the hypha bends round and forms a second spiral alongside the first. This process may occur several times, especially in cultures prepared by the *in situ* technique (Fig. 6). The occurrence of branched spirals does not appear to have been observed by previous writers.

By counting the number of coils (n) and measuring the inside diameter (d) of a spiral at right angles to the spiral axis, it is easy to calculate approximately the length of the hypha composing the spiral. When the successive coils of the spiral are in contact it may be assumed that each coil is a circle, and that the length (L) of the component hypha will be approximately given by the formula: $L = n\pi d$. d is taken as the inside diameter, as it is the inside or shorter side of the coil which will limit the extension of the hypha. In many spirals the successive coils are separated instead of being in contact; the length of the hypha as calculated from the formula will then be too low. The dimensions of seven hyphae which were selected at random

from three cultures are given in Table II, from which it will be seen that the hypha may be up to thirty times as long when extended as when coiled up in the spiral.

Table II. *The length of the hypha composing the spiral*

Isolate No.	Length of spiral	No. of coils	Inside diameter	Length of hypha (calculated)	Length hypha / Length spiral
	μ		μ	μ	
306	13	6	11	250	19 : 1
306	26	15	15	760	29 : 1
306	39	10	15	500	13 : 1
306	16	7	10	240	16 : 1
306	33	12	12	500	15 : 1
377	21	14	8	350	17 : 1
549	48	28	8	700	15 : 1

The number of spirals developed upon a mycelium is very great. On a medium prepared by diluting Sabouraud's maltose-peptone agar with nine times its volume of plain agar the spirals were relatively sparse and could be counted. A square millimetre of the surface of a mature colony on this medium was estimated, by counting the spirals visible on a small area under a low-power objective, to bear about five hundred spirals. In various isolations and on other media considerably different figures are likely to be found, but the number of spirals produced is certainly great.

Asymmetry of the spirals

In the description of the development of a spiral given above it was noted that as the tip of the hypha grows along the curved path which constitutes the first turn of the spiral, the tip becomes displaced to one side so that, instead of meeting the previously formed part of the hypha, it passes to one side of the older part of the hypha. An asymmetrical structure is thus produced, and all further growth of the hypha perpetuates this asymmetry. Further, it was noticed that, with very rare exceptions, the growing tip always passes to one particular side of the base of the spiral, so that the asymmetry of the resulting spiral is counter-clockwise.* That this counter-clockwise structure is not merely an attribute of one culture is shown by the list in Table III of isolations derived from various sources, in all of which counter-clockwise coiling was the rule.

* The sense in which the term "counter-clockwise" is here used is defined below in a section on the terminology of spiral structure. The meaning may be illustrated by saying that counter-clockwise spirals of *T. gypseum* resemble the counter-clockwise spirals of the scarlet-runner or *Convolvulus*, but differ from the clockwise spirals of the hop and honeysuckle among the twining plants.

Table III. *Cultures bearing spirals with counter-clockwise asymmetry*

Species	Source
Trichophyton gypseum	(144) *Sycosis parasitaria*, Manitoba
	(306) *Tinea corporis*, Manitoba
	(376) *Kerion Celsi*, Manitoba
	(377) *Kerion Celsi*, Manitoba
	(549) *Tinea corporis*, Manitoba
Trichophyton asteroides	American Collection of Type Cultures
Trichophyton granulosum	American Collection of Type Cultures
Trichophyton (Epidermophyton) interdigitale	Dr E. Muskatblit, New York
	(250) *Tinea pedis*, Manitoba
	(564) *Tinea pedis*, Manitoba
	(615) *Tinea pedis*, Manitoba
	(624) *Tinea pedis*, Manitoba
	(635) *Tinea pedis*, Manitoba
Trichophyton persicolor	National Collection of Type Cultures, London

The counter-clockwise structure was first observed in cultures on Sabouraud's maltose-peptone agar. To see whether nutrient substances influenced the direction of coiling, several strains were tested on a variety of other media, but no alteration was observed in the direction of twist. The media used were: (1) Sabouraud's maltose-peptone agar diluted with nine times its volume of plain agar; (2) Difco "Sabouraud's dextrose agar"; (3) Sabouraud's maltose-peptone agar; (4) wheat-flour extract agar; (5) whole-wheat flour paste; (6) human hair in moist tubes; (7) *in situ* cultures on naturally infected human hair; (8) sterilized horse dung; and (9) sterilized cow dung. On Czapek's solution-agar growth was poor and spirals were not produced.

External factors did not appear to affect the structure. Counter-clockwise spirals developed as normally in cultures grown in total darkness from the time of inoculation, as in those which were exposed to the usual diurnal alternation of light and darkness in the laboratory. The spirals also appeared to be insensitive to gravity, as the counter-clockwise rule held whether the spiral had grown vertically upwards, downwards, horizontally, or in intermediate positions.

The effect of temperature cannot be dealt with in the same way. When strains of *T. gypseum* and *T. interdigitale* isolated in Manitoba were grown on Sabouraud's maltose-peptone agar in the incubator at 37° C. spirals were not observed. This suppression of the formation of spirals at body temperature perhaps gives a clue to one of the factors responsible for the differences in the morphology of *T. gypseum* in its saprophytic and parasitic phases.

All the isolates examined so far have shown counter-clockwise spiral structure. Exceptions have been in the occasional occurrence of a few clockwise spirals in certain cultures in which, however, the majority of spirals were counter-clockwise. These clockwise spirals

are rare, and were observed in only two cultures of *T. interdigitale* isolated in Manitoba. In one of these cultures, out of 137 spirals carefully examined with the oil-immersion lens, only eight (less than 6 per cent) showed clockwise structure. In the other culture the proportion observed was only 3 per cent. It is possible that all cultures tend occasionally to produce clockwise spirals. It should be remarked that the clockwise spirals were well formed and normal in appearance. Apparently their production depended not so much on the breaking down of the normal mechanism for producing counter-clockwise spirals, as on a complete reversal of that mechanism. Perhaps this phenomenon will one day be explained as due to the local occurrence in the mycelium of a stereo-isomer of a normal molecule.

Experiments on the properties of the spirals

The properties of spirals were studied experimentally by means of a simple micromanipulator consisting of a glass needle or hair attached at an angle of about 30° to a horizontal glass rod. The glass rod was mounted in a cork in place of the objective of a microscope. The focusing device of this microscope provided a delicate vertical movement of the needle. A Petri-dish culture of *T. gypseum* was placed for observation on the stage of another microscope. By use of the mechanical stage of the observation microscope, motion in two other directions was possible. By means of this micromanipulator the tip of the glass needle or hair could be made to touch any part of the mycelium in the Petri dish. This simple micromanipulator was suggested to one of us in 1930 by Dr T. R. Vernon.

In one experiment the properties of spirals were studied by touching them with glass needles of 30–40μ diameter which had been cleaned in alcohol and ether in order to free them from grease. If ordinary straight vegetative hyphae were touched with a needle, hypha and needle adhered. When by means of the micromanipulator the needle was withdrawn the adhesive force was sufficient to deform the needle. However, on withdrawing the needle still further the adhesion was broken and both hypha and needle swung back to their former positions. Adhesion between hypha and needle was insufficient to break the hypha free from the mycelium.

If the needle were made to touch either the tip of a spiral or the middle of a coil the same phenomenon was observed, but owing to the uncoiling of the spiral the needle had to be withdrawn further before the hypha was fully extended and adhesion broken. The spiral hypha when drawn out was seen to be flattened and appeared as a chain of arcs. This form would be expected from the flattening of the hypha already noted above. When adhesion between hypha and needle had been broken the hypha usually regained its spiral form completely.

The peculiar flattening of the spiral hypha in a plane at right angles to the axis of the spiral no doubt has a function in restoring the hypha to the original shape after it has been temporarily deformed. On account of this flattening there will be more resistance to any deformation of the curvature of the hypha, and less resistance to distortion of the coil in the direction of the axis of the spiral.

If the needle was made to touch a number of coils of a spiral, or to touch several adjacent spirals at the same time, events followed a different course. On withdrawing the needle adhesion was sufficient to detach a group of spirals, together with conidiophores and conidia (aleuriospores), from the mycelium, and to leave them adherent to the needle.

In place of glass needles human hairs were used in repeating these experiments, and similar results were obtained. The general effect of the spirals may be described as presenting a large adhesive surface to foreign bodies and so tending to break off a portion of the mycelium.

The micromanipulator method was also applied to cultures of *Microsporum felineum*, on whose mycelium as a rule no spirals are present. The hyphae in this species adhered to the needle only feebly, and repeated attempts failed to detach a single hypha or fuseau by adhesion to the needle.

The use of microscope and micromanipulator was not necessary in order to demonstrate the adhesive properties of the mycelium of *Trichophyton gypseum*. If an ordinary steel needle be touched lightly to the granular surface of a colony growing on agar and then withdrawn it can be observed with the naked eye that a tuft of white hyphae and spores about a millimetre in diameter has adhered to the tip of the needle. On the other hand, a needle touched to the surface of a culture of *Microsporum felineum* does not bring away a tuft of hyphae but only serves to compress together the hyphae of the culture. It is therefore obvious that a mycelium of *Trichophyton gypseum* has adhesive properties which are not possessed by a mycelium of *Microsporum felineum*. That this adhesiveness in *Trichophyton gypseum* is not due solely to the presence of spirals is shown by the fact that a "pleomorphic" culture of the species which had ceased to produce spirals still exhibited some adhesiveness when touched with a needle. The vegetative hyphae of *T. gypseum* appear to be sticky. There is nothing at present known to suggest that the spirals are more sticky than the vegetative hyphae, but the presence of spirals on the mycelium must ensure that any foreign body such as an animal touching the mycelium comes into contact with a very large area of sticky surface and leaves with a relatively large inoculum consisting of spirals, hyphae and spores firmly anchored. The spirals appear to function as local concentrations of a large quantity of sticky mycelium.

The tarsi of house flies which were induced to walk over cultures

of *T. gypseum* at once became coated with white masses of hyphae. The flies were then made to walk across the surface of sterile agar slants, and on incubating these, cultures of *T. gypseum*, recognizable by the characteristic spirals and other organs, were obtained.

There seems no reason to believe that the spirals of *Trichophyton* are functionless; rather they must be looked upon as structures serving to attach a large number of the spores which are produced while the organism is growing in its saprophytic phase to any animal which happens to come into contact with the mycelium.

Discussion

The fact that a clockwise or a counter-clockwise asymmetry is usually specific for twining plants among the Phanerogams has long been familiar to botanists. Spirally twisted organs of various kinds also occur in certain fungi, but in this group, where microscopic examination is necessary to elucidate the spatial structure of the spiral, less attention has been paid to the subject. Spiral structure in the genus *Trichophyton* is worthy of study, since differences in such a clear-cut character, if found between distinct species, might be of value for the easy identification of members of this difficult genus. A method of determining the spatial structure of microscopic objects by differential focusing has been worked out and is presented as an Appendix to this paper in the hope that observations will be made on Dermatophytes and other Thallophyta by other workers. In this connexion it will be necessary to discuss the confused nomenclature of spiral structure, and to define the terms used, since studies of spatial structure are misleading if expressed in ambiguous terms.

Nomenclature

The plane spiral. For a plane spiral, whose coils lie in one plane as illustrated in Fig. 9, there is only one possible spatial structure. The apparent existence of two forms is due merely to the position of the observer, as may be shown by examining Fig. 9 through the back of the page against the light. Among fungi spores in the form of a plane spiral occur in certain members of the Helicosporeae.

Three-dimensional spiral or helix. For a three-dimensional spiral there are two possible spatial structures which differ absolutely irrespective of the position of the observer. The nomenclature of these forms is confusing, as workers in different fields of science, or even in the same field, have used terms in opposite senses. This confusion is discussed by Jackson (1916).

For anatomical reasons the head of a screw which is being driven into a piece of wood can be rotated by the right hand with more force in a clockwise direction than in a counter-clockwise direction. The

common wood-screw is accordingly threaded so that when twisted in a clockwise direction by the right hand it will be driven forward away from the operator. Such a thread, which is illustrated in Fig. 10, is called by the engineer a right-handed thread, and this logical terminology appears to be followed consistently by zoologists and human anatomists, and also by certain botanists. Unfortunately many botanists, including Darwin, Hooker and Strasburger, applied the term right-handed to twining plants whose spatial structure corresponds with the engineers' left-handed thread. The confusion which already exists in the nomenclature is illustrated in Table IV.

Table IV. *Differences in nomenclature of spiral structure*

Engineers, zoologists, anatomists and earlier botanists		Many later botanists
Right-handed	=	Left-handed
Dextrorse	=	Sinistrorse
Dextral	=	Counter-clockwise
	=	Anti-clockwise
	=	Against the sun*
Left-handed	=	Right-handed
Sinistrorse	=	Dextrorse
Sinistral	=	Clockwise
	=	With the sun*

* These terms are ambiguous as they have opposite meanings in Northern and Southern Hemispheres.

Inspection of Table IV shows that if a structure is to be described as right-handed or left-handed it is essential to state clearly what system of nomenclature is being followed. In the present work the terms *clockwise* and *counter-clockwise* have been adopted as being less likely to lead to confusion than *right-handed* and *left-handed*. The definition of clockwise and counter-clockwise structure given in the following paragraph has been based on the concept of an observer viewing the spiral as a static structure. In order to make the definition of general applicability all ideas of growth or movement of the spiral have been avoided.

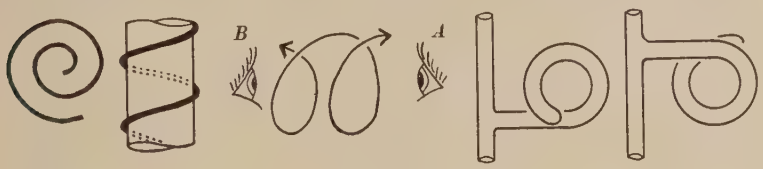
Definition. An observer situated outside the spiral, and on its axis, describes the structure as *clockwise* if the end of the coil nearer him points in the direction of rotation of the hands of a clock. Conversely, when the end of the coil nearer him points in a direction contrary to rotation of the hands of a clock the spiral is said to be counter-clockwise.

Fig. 11 illustrates a spiral of which the observer situated at *A* regards the end of the coil nearer him as pointing in the clockwise direction. The end of the coil farther from him, at *B*, will appear to him to point in the opposite direction (counter-clockwise). It is therefore important that the convention should be applied only to the

direction in which the end of the thread nearer the observer points. However, a second observer situated at *B* will regard the end of the coil at *B* as clockwise, and that at *A* as counter-clockwise, simply because he is using his own clock as reference and not that of the observer at *A* seen in reverse. Therefore by following the convention an observer situated at either end of the spiral will reach the same conclusion with regard to its spatial structure.

Spiral organs in the fungi

Spiral organs occur in a variety of fungi. Both clockwise and counter-clockwise forms occur, but, so far as attention has been paid to the subject, the evidence indicates that for any particular species the spatial structure is constant. Whether structural similarity ex-



Figs. 9–13.

tends to the wider groups it is at present impossible to say. The following examples indicate the constancy of spatial structure within the species.

Drechsler (1919) observed that rotation in the formation of spirally coiled sporogenous hyphae in the genus *Actinomyces* is specifically "sinistrorse" [counter-clockwise] or "dextrorse" [clockwise]. Out of sixteen species in which the condition could be ascertained with certainty, eleven were counter-clockwise, and five were clockwise. Drechsler drew attention to the absolute constancy with which a species adheres to one kind of rotation.

In the Myxomycete genera *Trichia* and *Hemitrichia* the capillitium threads in the sporangium have one or more helically coiled bands of thickening on the cell wall. These show a consistently clockwise structure and are so figured by Lister (1925). It is, however, interesting to note that an aberrant specimen of *Hemitrichia leiotrichia* is recorded by Miss Lister with dextral [counter-clockwise] spirals.

In Zopf's (1881) monograph of the genus *Chaetomium* spirally coiled hairs are depicted around the ostiole of the perithecium in several species. Definite statements as to their spatial condition are lacking, except that in *C. crispatum* Fuckel the coils are said to alternate in direction along each hair. The species must be almost unique in this respect. Zopf's carefully drawn figure of *C. spirale* clearly shows a

preponderance of counter-clockwise spirals. A culture of *C.* ? *spirale* which had been isolated from Manitoban soil and supplied by Dr G. R. Bisby when examined by us showed only counter-clockwise perithecial hairs.

A study of the figures in Linder's (1929) monograph of fungi belonging to the Helicosporeae indicates that the condition differs between different species. A specimen of *Helicoon ellipticum* (Pk.) Morgan in the Manitoba Agricultural College Herbarium was found by us to possess counter-clockwise spores.

These observations indicate that any particular collection or isolate of a fungus shows a high degree of uniformity in the spatial structure of its spiral organs. Further, this uniformity commonly extends to other individuals of the same species. It appears probable that spiral spatial structure is specific except for occasional aberrations. There is need for more data on this subject, and it is essential that the data should be expressed in unambiguous terms.

The spiral hyphae of *Trichophyton* agree with similar organs observed in other fungi in showing a high degree of uniformity of spatial structure. Further, in the possession of predominantly counter-clockwise spirals they resemble *Chaetomium spirale*, *Helicoon ellipticum* and certain *Actinomyces*, but differ from the Myxomycetes *Trichia* and *Hemitrichia*.

Summary

The spiral hyphae of *Trichophyton* are believed to be organs of attachment playing a part in the dissemination of the fungus and in infecting animal hosts from saprophytic sources. Mature spirals are empty, dead hyphae, flattened in a plane at right angles to the axis of the spiral. They may be branched.

The spirals observed in five isolates of *T. gypseum*, six of *T. interdigitale* and one each of *T. asteroides*, *T. granulosum* and *T. persicolor* were found to be counter-clockwise. No isolates were found with clockwise spirals predominating. Occasional clockwise spirals were however found in some isolates of *T. interdigitale*.

When deformed by mechanical contact a spiral regains its original shape, and it is probable that this process is aided by the flattening of the spiral hypha.

The mycelium of *T. gypseum* is sticky and tends to adhere to objects which come into contact with it. This stickiness is not shared by *Microsporum felineum*. When a sufficient number of adjacent hyphae or coils of a spiral adhere to an object they can break away from the mycelium. It is concluded that the function of the spirals is to present a large adhesive surface to any foreign body. Thus a relatively large inoculum is attached to an animal which touches the saprophytic mycelium.

APPENDIX

The determination of spiral structure by differential focusing

The method of determining the spiral structure of microscopic objects by differential focusing consists in observing the object under the microscope at such a magnification that only a portion of the spiral appears in sharp focus at one time. Then by differential focusing the spatial relations of the parts can be made out.

Two principal positions are encountered in examining spiral structures under the microscope: (1) when the observer is situated on the axis of the spiral; and (2) when the observer is situated at right angles to the axis of the spiral.

(1) *Observer situated on the axis of the spiral.* By focusing on the nearer end of the coil the structure of the spiral can be read off at once by following the definition given on p. 108. When the object is a growing structure attached at one end, such as a fungal hypha, two conditions arise according as the structure has grown towards or away from the observer.

When the spiral has grown vertically upwards towards the observer no difficulty will arise in applying the definition (Fig. 12). On focusing downwards the end of the coil nearer the observer first comes into focus, and from this the structure of the spiral can be determined at once. On focusing successively lower planes the further end of the coil is reached, and is found to point in the direction opposite to the nearer end.

When the spiral has grown vertically downwards away from the observer, the attached end of the coil which is nearer the observer comes into focus first on focusing downwards (Fig. 13). According to the definition the direction in which this end points is taken as giving the structure of the spiral; usually this direction will be opposite to that in which growth has taken place during the formation of the structure; this fact is, however, irrelevant according to the definition. On focusing down farther the tip of the spiral comes into focus and will be found to point in a direction contrary to that from which the structure is named. An observation of the more distant end of the spiral can sometimes be made use of when a good focus cannot be obtained on the nearer end.

(2) *Observer situated at right angles to the axis of the spiral.* When the observer looks at the spiral radially or "sideways-on", it is not necessary to find the ends of the coil in order to determine the structure. To an observer situated radially with respect to the axis the coils will appear to cross the axis at some angle other than a right angle. Further, if the coils lying between the observer and the axis cross the axis at an angle θ° on the right side of the axis, then the coils lying

below the axis will make an angle $\theta°$ on the left side of the axis as shown in Fig. 14, where a portion of a counter-clockwise spiral is illustrated. On focusing down slowly on to such a spiral the portions of the coil *ab* and *cd* in the plane nearer the observer come into focus first; on focusing below the axis the portion *bc* comes into focus. By considering the axis as running north and south we can express the structure by saying that in a counter-clockwise spiral the coils nearer the observer run north-east and south-west relative to the axis, while on focusing down it can be seen that the farther parts of the coil run north-west and south-east. Conversely, in a clockwise spiral the coils above the axis run north-west and south-east, while those below the

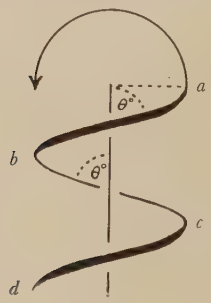


Fig. 14.

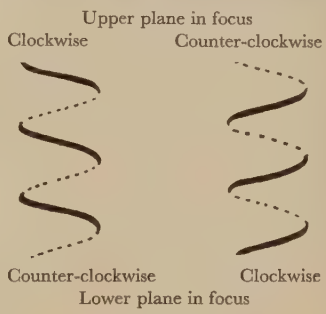


Fig. 15.

axis run north-east and south-west. The relations between the nearer and farther planes of focus in both clockwise and counter-clockwise spirals is compared diagramatically in Fig. 15, in which those parts of the spiral which are in focus in each plane are represented by thickened lines. The compound microscope rotates the image through 180° in the plane of the field, and consequently the microscope gives a true picture of spiral spatial structure. By the method of differential focusing here described the spatial structure of a spiral can be determined by observations made on a very small portion of the spiral examined in any position.

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SOME OBSERVATIONS ON THE OCCURRENCE OF *FUSARIUM CULMORUM* ON WHEAT

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In published work dealing with the occurrence of *Fusarium culmorum* on cereals most attention has been devoted to the status of this fungus as a parasite. Simmonds (7), Bennett (1), Russell (5), Geach (3) and others have recorded it as causing seedling blight, foot rot, whiteheads or ear blight of oats and wheat in the field, and have proved its pathogenicity by controlled inoculation experiments. In recent years, however, references to its occurrence either as a weak secondary parasite, or under circumstances where its parasitic action has been doubtful, have been more numerous. One of the most interesting of these is the work of Broadfoot (2) in which isolations from many thousands of wheat plants taken at random from rotation plots yielded *F. culmorum* (either alone or in combination with other fungi and bacteria) to the extent of from 20 to 60 per cent in different plots. Simmonds (7) and Sanford & Broadfoot (6) have also isolated *F. culmorum* from stubble. The latter workers compared the virulence of many isolates and found that most of them were only weakly pathogenic. Apart from this recent work in Canada the information available on the weakly parasitic and saprophytic life of *F. culmorum* on cereals is comparatively scanty. It therefore seems worth while putting on record the results of isolations made from healthy wheat plants collected in England.

Isolations made at Rothamsted about harvest time in 1934 showed that *F. culmorum* was present on most of the roots and stem bases of wheat plants affected with whiteheads. However, control isolations made from healthy plants with full ears of grain revealed that *F. culmorum* was often present on the roots and stem bases of these plants also, so that no conclusion could be drawn as to the parasitic action of the fungus where it had been isolated from the whitehead plants.

In the following year, with a view to obtaining some idea of the progressive invasion of the roots and crowns of wheat plants by fungi, isolation work was begun about the time the crop was in flower, and periodic isolations were made as the crop ripened. The material for this study was obtained from three wheat fields, one at Rothamsted, one at St Albans and one at Cambridge (Table I).

The crops on these fields were among the healthiest in the districts,

Table I. *Cropping data for fields examined*

	Soil	Variety	Sown	Reaped	Rotation	Yield per acre
Rothamsted	Clay with flints, *p*H 7·8	Victor	26 Oct. 1934	6 Aug. 1935	1932 kale 1933 barley 1934 beans	
St Albans	Clay with flints, *p*H 7·2	Victor	27 Sept. 1934		Temporary grass ley 1934 oats	50 bushels
Cambridge	Heavy gault clay, *p*H 7·9	Yeoman I	29 Oct. 1934	30 Jul. 1935	Temporary grass ley	58 bushels

and showed no evidence that seedling diseases had been present. At intervals of about a fortnight ten samples of about a dozen plants were taken from each crop. These samples were taken at intervals of ten paces and between ten and twenty paces in from the edge along the best side of the field. From each sample the healthiest plant was chosen, and four crown pieces and five root pieces cut out at random. These were rinsed in alcohol, sterilized two minutes in 1:1000 mercuric chloride, washed in sterile water, and plated out on potato-dextrose agar, the crown pieces and root pieces being plated in separate dishes. The last isolations were made from the stubbles after the crops had been cut. A summary of the results is given in Table II.

Table II. *Isolations of* Fusarium culmorum *and other fungi from healthy wheat plants*

				Number of pieces yielding		
Field	Date of collection	Part of plant	No. of pieces	*Fusarium culmorum*	other *Fusarium* spp.	other fungi and bacteria
Rothamsted	26 June	Crown	40	0	4	29
		Roots	50	5	2	36
	7 July	Crown	40	4	1	31
		Roots	50	2	2	41
	22 July	Crown	40	3	5	32
		Roots	50	2	3	45
	6 Aug. (stubble)	Crown	40	1	0	35
		Roots	50	0	1	46
St Albans	1 July	Crown	40	0	0	17
		Roots	50	1	0	15
	15 July	Crown	40	1	5	33
		Roots	50	5	3	36
	29 July	Crown	40	14	3	21
		Roots	50	10	2	38
	13 Aug. (stubble)	Crown	120	42	2	66
		Roots	150	34	0	106
Cambridge	12 July	Crown	40	13	0	23
		Roots	44	14	0	21
	26 July	Crown	40	11	4	25
		Roots	150	14	5	75
	20 Aug. (stubble)	Crown	120	90	0	4
		Roots	150	130	0	6

It will be seen that *F. culmorum* was isolated from a few specimens in every field at the time of the first sampling. In the Rothamsted field it did not increase in amount as the season advanced; in fact, it decreased. In the St Albans and Cambridge fields, on the other hand, it increased markedly, and in each, seven of the ten plants taken before harvest had the fungus on stem base or roots. In the field at St Albans thirty stubble samples taken at random over the field two weeks after harvest showed 70 per cent infection with *F. culmorum*, and in the Cambridge field the fungus was present on every one of fifty random stubble samples taken three weeks after harvest.

In spite of the appreciable amount of *F. culmorum* present on the roots at harvest time the crops gave excellent yields. The one at St Albans yielded 50 bushels to the acre, and the one at Cambridge 58 bushels. In the latter there were fourteen sacks of head corn and only two-thirds of a sack of tail corn, so that the grain had filled remarkably well. A sample of wheat from this crop took the Gold Cup at the Baker's Exhibition, London, for the best sample of milling flour.

To determine whether the strains of *F. culmorum* obtained were weak and almost non-pathogenic, isolates from the different localities were multiplied on corn-meal sand, and pathogenicity tests on wheat seedlings in the greenhouse were carried out in the usual manner. When the seedlings were washed out at the end of five weeks there was appreciable attack on the roots by all the strains of *F. culmorum* used. The average disease rating (4) for thirteen isolates of *F. culmorum* was 21·0 (ranging from 9 to 40), that for three isolates of *Ophiobolus graminis* was 99·0, and that for the controls was 7·5. Sanford & Broadfoot (6) have drawn attention to the difficulties encountered in greenhouse tests for pathogenicity when done by the usual methods. Not much reliance was therefore placed on these tests, and the question of the relative pathogenicity of strains of *Fusarium culmorum* in England was left for more detailed investigation in the future.

Although isolation work was not commenced quite early enough, and was not done on a sufficient number of samples to give a complete picture of the activity of *F. culmorum* during the season, it is evident that the fungus must have been present in the soil from which these healthy wheat plants were taken, exerting no appreciable parasitic effect and entering the root systems, along with other fungi, only as the roots began to lose vitality after flowering of the crop. There was then a still further development of the fungus on the stubble, after the crop had been cut. This is in contrast to fairly numerous cases reported, especially from the north of England, of appreciable damage to wheat by the fungus *F. culmorum*. The soil or other factors which favour parasitic action by this fungus during the earlier stages in the growth of cereal plants are at present very imperfectly understood, but

it may be significant that these three instances in which no apparent injury was done were all on slightly alkaline clay soils.

The assistance of Miss E. Holmes and of Dr M. Fernando with portions of the cultural work, and of Dr W. A. R. Dillon Weston in the collection of samples from Cambridge, is gratefully acknowledged. Dr F. J. Greaney shared in the work described in the paper while he was a temporary worker at the Rothamsted Experimental Station. He is a member of the staff of the Dominion Rust Research Laboratory, Winnipeg, Canada.

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SYMPOSIUM AND DISCUSSION ON LABORATORY TECHNIQUE FOR EVALUATING FUNGICIDAL PROPERTIES

Held on 18 *January* 1936, *in the Botanical Department, University College, Gower Street.*
The President, Mr F. G. Gould, *in the Chair*

I. PROTECTIVE FUNGICIDES AGAINST APPLE SCAB (*VENTURIA INAEQUALIS*)

By H. B. S. Montgomery and M. H. Moore
East Malling Research Station

I. The aims underlying the work

Venturia inaequalis was chosen as the test fungus because the need for the present investigation at East Malling arose out of field-spraying experiments designed to determine control measures for the disease caused by this fungus.

The test fungicide was Bordeaux mixture (proportion of copper sulphate to hydrated lime as 2 is to 3). This selection may be criticized in that this spray fluid is rather complex chemically and its action is not fully understood. Yet it was selected because of, rather than in spite of, that complexity. Something, at least, is known of its action and its properties, and if a laboratory method is evolved and built up by using a spray fluid of this nature, many of the difficulties inherent in the process will have been encountered and, in part, overcome.

The criterion selected was a simulation of field conditions as far as possible, not by exact reproduction of the many factors involved, but by standardization of such amongst them as lent themselves to standardization.

Hence, it was necessary to produce:

(*a*) A standard surface on which to carry out the tests.

(*b*) A standard, dry spray deposit, to which scab spores are added.

(*c*) The thinnest complete film of spray fluid practicable (Serge Héranger (1) considers that $\frac{1}{10}$ mm. is the lower limit of thickness of film that can be obtained by spraying).

(*d*) A standard process by which the spray deposit can be washed, as it is washed on leaves by rain, dew, etc.

(*e*) A suspension of spores standardized for quantity and quality.

(*f*) Standard conditions (temperature and moisture) for germination.

(*g*) A method of evaluating results.

II. Methods of procedure

(*a*) *Surface.* Apple leaves differ from one another even within a variety and they are not, at present, available all the year round. Glass slides, 3 × 1 in., chemically cleaned, are therefore used as offering the best available and convenient surface where action between the surface and the spray deposit will be infinitesimal or nil, and the influence of foreign matter can readily be eliminated. Three circles, each of exactly 15 mm. diam. (arbitrary), are scratched on each slide, by revolving the slide in a lathe in which a glass-cutter's diamond is mounted.

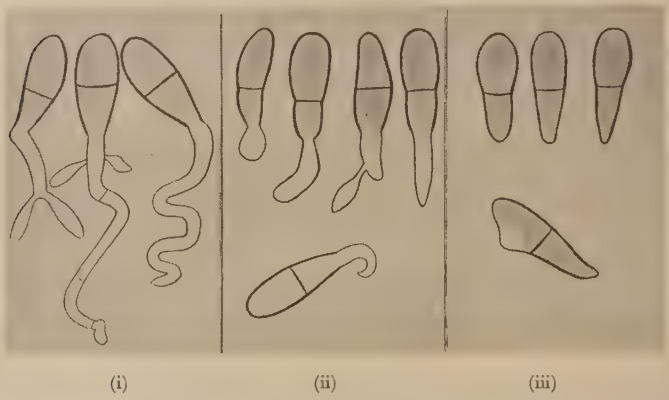
(*b*) and (*c*) *Spray fluid and the dry deposit.* Bordeaux mixture of known strength is applied to the area enclosed by each ring at the rate of 0·015 c.c. per ring. The fluid is delivered from a specially graduated, fine-bore glass pipette, and it is spread to the edge of the ring, where necessary, with a glass needle. The thickness of the fluid film is thus approximately $\frac{1}{10}$ mm. (*vide* Héranger (1)). The slides are then air-dried and stored at 20° C. until next day.

(*d*) *Washing.* Rain water is variable in nature, and the use of running distilled water is extravagant and inconvenient to manipulate. The slides bearing the spray deposit are therefore placed face downwards on a glass rack in a bath of fresh distilled water, and rocked horizontally for given periods by mechanical means. Slides bearing a similar spray deposit are left unwashed as controls.

(*e*) and (*f*) *Spore suspension and incubation.* The fungus was cultured on ordinary malt-agar plates and on cheese-cloth wicks soaked in malt extract (2), but neither method proved suitable for readily providing supplies of free spores (conidia). Eventually, cultures were developed on agar films and on pieces of sterilized apple wood, and both methods yielded free spores readily. Cultures are kept at constant temperature and with the minimum moisture necessary to prevent drying out. A spore suspension is usually obtained readily by washing the surface of the agar film, or of the piece of wood, with sterile distilled water. Nutriment tends to be extracted in varying amounts by the distilled water, both with agar films and apple-wood cultures. Methods of obviating this disadvantage have recently been tested. As a result, the use of agar films has been abandoned, for portions of the agar float in the spore suspension and cannot easily be removed. Suspensions obtained from apple-wood cultures can, however, be centrifuged and washed free from nutriment. After centrifuging, the supernatant liquid is poured off, sterile distilled water is

added to the concentrated spores, and the process is repeated until the spores ultimately remain suspended in pure distilled water.

It has been found necessary to obtain spore suspensions for tests from young cultures, for older cultures give mixtures of spores of different ages. It is now the practice to choose for the supply of spores cultures, 10–12 days old, which readily yield in suspension at least fifty spores per 2 mm. field. The suspension is ultimately regulated to contain about eighty spores per 2 mm. field. The spore suspension is put on the dry spray deposit on the slides at 0·04 cc. per ring by means of another specially graduated, fine-bore glass pipette, similar to that



Conidia of *Venturia inaequalis* showing the three groups into which the spores are classified when evaluating results of tests. (i) germinated, (ii) inhibited after initial germination, (iii) ungerminated.

used for the spray fluid. The same amount of spore suspension is also put within rings on slides without any spray deposit, and these act as controls and keep a check on the quality of the spores. The suspension is spread to the groove with a glass needle, and the slides are then placed in moist chambers (Petri dishes each containing a pool of distilled water to provide a saturated atmosphere) and incubated at constant temperature (20° C.).

(*g*) *Evaluation of results.* When the spores have germinated, but before growth has developed far enough to produce secondary conidia, germination is recorded by counting fields of spores more or less at random within each ring. The results are expressed at present in terms of spores (i) *germinated*—germ tube longer than spore, (ii) *inhibited after initial germination*—germ tube up to length of spore, (iii) *ungerminated*—no germ tube (see figure). It is usual on control slides to find at least 90 per cent of the spores in the first group.

III. Results

Up to the present, the results obtained have been used to detect weaknesses in the method and thus to develop it, rather than to make comparisons of fungicidal efficiency. Many of the results could not reasonably be compared, for they were obtained under conditions that are now known to have been variable. However, certain points have emerged that are worth recording:

(*a*) Bordeaux mixture at very low concentrations is toxic to scab spores. A mixture containing as little as 0·0125 per cent copper sulphate (or, in practical terms, 2 oz. per 100 gallons of spray, which is one-sixty-fourth of the concentration commonly used in commercial spraying) virtually prevents germination even after the dry deposit has been washed continuously for at least an hour.

(*b*) The age of the culture, and the conditions of temperature and humidity under which it was grown, largely determine the quantity, and possibly the quality, of the spores readily liberated in distilled water. The later experiments have shown that there may also be certain other issues involved.

(*c*) The susceptibility of scab conidia to killing by Bordeaux mixture is governed by the presence or absence of nutriment in the suspension.

IV. Future developments

It is anticipated that further work will be necessary before the method is perfected, while several of the tentative conclusions already reached need confirmation by more tests. The method will then be used for comparison of the fungicidal properties of new products (as yet not tested, or only partially tested, in the field) with those of the better-known sprays already in use commercially. Many points concerning the effect on fungicidal efficiency of the addition of spreaders, insecticides, or substances designed to obviate spray damage should, theoretically at least, be susceptible of evaluation by the use of this technique. It is often not easy to gauge by field trial the influence of these addenda on fungicidal value, for an apparently direct influence in these circumstances may be indirect, and due to differences in the physical properties of the spray fluids.

It is not unreasonable to hope that some light may be thrown on the fundamental action of certain spray fluids, either directly, or by leading up to a line of investigation through other channels. Certain questions with a possible bearing in that direction have already arisen during the present work with Bordeaux mixture.

Ultimately, promising new fungicides, which have proved non-phytocidal in small trials in the field, will be tested on trees growing in pots under standard conditions in the greenhouse. This will involve the development of a satisfactory method of inoculating leaves

and fruits with the fungus. The final stage will be the intercomparison of the most promising new or improved spray fluids by field trial.

The continuation of this work, begun in November, 1934, was made possible by the aid of a grant from Imperial Chemical Industries, Ltd., to whom the writers record their indebtedness.

[Some of the apparatus used in this work was exhibited during the meeting, and the method of washing the spray deposit on the slides was demonstrated.]

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II. THE EVALUATION OF PROTECTIVE FUNGICIDES WITH SPECIAL REFERENCE TO APPLE SCAB *VENTURIA INAEQUALIS*[1]

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III. PHYSICO-CHEMICAL ASPECTS

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The status of physico-chemical methods in the laboratory examination of any particular fungicide is determined by the extent of knowledge of the mode of action of that and related substances. Once the fungicidal properties of the substance have been correlated with specific chemical and physical properties, laboratory testing is reduced to the evaluation of those properties. From the results obtained, the performance of the substance under field conditions can be foretold and an examination by biological methods is rendered unnecessary. For example, standardization by biological methods of lime sulphurs can be replaced by the analytical determination of content of polysulphide sulphur.

It is evident that the trustworthiness of the conclusions derived from the physico-chemical examination of the substance depends upon experience of the field performance of related compounds. For this reason, it is most important that the biological tests of any new product should have collateral physico-chemical tests designed not only for the purpose of defining the new product but for the eventual elucidation of its mode of action. The usefulness of such supplementary laboratory tests is illustrated by the following results obtained in an investigation of the protective fungicidal efficiency of

[1] See these *Transactions*, vol. xx, 304–9.

certain copper derivatives, Potato Blight being used as the test organism. As a working hypothesis it was assumed that the efficiency of the spray will be dependent upon the retention on the foliage of a copper-containing deposit from which the active fungicide is slowly formed. It follows that the protective efficiency is determined both by retention factors and by availability factors, and to separate these two sets of factors analytical studies were made of the sprayed potato foliage and the total amount of copper present at intervals after application was determined. Simultaneously, estimates of blight infestation were made in the field, with the results recorded in Table I.

The partial correlation between protective efficiency and retention is shown and differences attributable to availability can be determined. For example, comparing the relative efficiencies of cupric oxide and of cuprous oxide, the indications are that cupric oxide is better retained whereas cuprous oxide has the greater availability. In the dry

Table I

1934 trials			1935 trials		
Spray	Average estimate blight infestation	Average copper retention	Spray	Average estimate blight infestation	Average copper retention
Cotton-seed-Bordeaux	3·78	0·53	Cupric phosphate	4·40	0·38
Bordeaux	3·94	0·38	Cupric oxide	4·80	0·38
Cuprous cyanide	4·06	0·18	Cupric silicate	5·00	0·30
Cuprous oxide	4·44	0·18	Copper sulphite	5·20	0·12
Cupric oxide	5·33	0·22	Cupric oxychloride	5·53	0·20
Colloidal Burgundy	5·39	0·15	Burgundy Paste	5·43	0·19
Petroleum-Bordeaux	5·50	0·31	Cuprous oxide	5·50	0·19
			Cuprous cyanide	5·67	0·30
			Bordeaux	6·30	0·52
Significant difference	0·846	0·05		0·318	—

season of 1934 the latter factor was predominant and cuprous oxide proved the superior protector; in the wet 1935 season, the greater amount of cupric oxide retained overshadowed its lower availability and cupric oxide proved the better fungicide. Conclusions of this character must obviously be confirmed by further trial and they are instanced only for the purpose of demonstrating the value of physico-chemical tests as supplements to field trials. It is not, at this stage, suggested that they are essential and it may be possible to subject an unknown product *XYZ* to field trials and, if found satisfactory, to admit the product to the category of fungicides suitable for recommendation. Physico-chemical criteria play no part in such a scheme but, once the field trials are replaced by laboratory biological tests, physico-chemical considerations again require attention.

The simplest definition of a laboratory trial is one in which one or more of the factors affecting fungicidal efficiency which are variable in the field trial are maintained constant. This definition may be

illustrated by a brief description of Salmon's method of assessing direct fungicidal properties, which employs the hop Powdery Mildew as the test fungus. By using hop plants vegetatively propagated from one parent plant susceptible to the mildew and by selecting for treatment leaves and mildew patches in the same stages of active growth, the biological condition of the fungus and host plant is standardized and variations in their condition which may affect the response of the fungus to the fungicide are diminished. Further, the physical properties of the spray are modified so that it is able to displace air from and to wet the conidiophore mass. Finally, a sufficient amount of spray is applied to achieve complete wetting. In interpreting to practice the results obtained by this technique, it must be remembered that, as variations in the biological condition of the fungus and host plant have been eliminated, the results are of relative and not absolute value. Further, that the results are applicable only to sprays which have wetting properties sufficient to wet the conidiophores when applied in heavy amounts in the field.

The accuracy of the interpretation of the results of laboratory tests is dependent upon the correctness of the allowances made for the influence of variations in the factors held constant in the trial. This theme has been developed in a previous discussion (1) and it is only necessary to emphasize that the missing variables are always found to involve physico-chemical factors. The neglect of such criteria, which was conceivable in field trials, becomes highly dangerous in laboratory tests.

From the biological aspect, the laboratory trial is generally designed for the examination of the toxicity of the material to fungi. Fungicidal efficiency, although primarily dependent upon actual toxicity, is determined by other factors, of which the amount of material applied and retained by the fungus or plant surface may be selected as an example. In all laboratory methods of evaluating fungicidal properties, standard procedure is adopted for the application of the spray or dust under test. Moore and Montgomery spread a definite volume of spray over a standard area of surface; Marsh and Salmon both apply the spray under standard conditions to the surface held at right angles to the direction of the spray; Marsh spraying for a definite time, Salmon spraying copious amounts of a spray of good wetting properties. What assumptions do these various procedures involve and how are the results comparable with those of practical spray methods?

With the surface held at right angles to the direction of the spray it has been found (2) that, with solid-liquid systems akin to those encountered in spray practice, the whole of the spray applied is retained up to a certain amount dependent upon the particular liquid-solid system. Irrespective of the spray or surface, the amount re-

tained is equal to the amount applied until the volume present is such that the excess runs or drains off the surface. By the technique used by Marsh, therefore, standard amounts of spray are applied per unit area provided that the time of spraying be so selected that no run-off or drain-off of the spray occurs. The technique thus produces the same end-point as that of Moore and Montgomery except that the latter method makes no apparent provision for the possibility that excess of spray may be applied to the surface. In Salmon's method, excess of spray is deliberately applied and the amount retained will be determined by the wetting properties of the particular spray under test. But a requisite of the technique is that the wetting properties of the spray shall be sufficient to permit complete wetting. Under such conditions it has been shown that approximately constant amounts of spray are retained.

Salmon was concerned with direct fungicidal properties for which purpose only sprays of good wetting properties are suitable. The other techniques under discussion are applied to sprays of indifferent wetting properties such as are normally used as protective fungicides. With such sprays, the amounts retained upon foliage will be limited by the maximum amount retainable, an amount which has been shown to be greatly influenced by the physical properties of the spray. Thus upon a cellulose nitrate surface, the maximum retainable weight of water is of the order of 200 mg. per sq. in.; that of a solution of an effective wetting agent is of the order of 25 mg. per sq. in.

There is, however, a compensating set of factors, for the condition that the surface shall be held perpendicular to the direction of the spray is but rarely met in practice. If the surface be tilted to the spray it is found that only a proportion is retained, the proportion increasing with the wetting properties of the spray. When applied in limited amount, therefore, a spray of wetting properties similar to those of water will be retained in less amount than a spray of good wetting properties provided that, in neither case, does drip occur.

These considerations concern simple aqueous solutions, and with heterogeneous sprays, such as suspensions and emulsions, other physical factors come into play. Enough has been said to show the complexity of the apparently simple problem of how to apply the spray in the laboratory test. The exact reproduction of the spray deposit obtained in practice is not necessary provided that the effect of the factors held constant in the laboratory test be remembered in translating the results to practice. For this reason there is much to be said for the simplest technique which will give consistent results and of which the significance in relation to field technique is understood.

The method of application is but one example of the physico-chemical considerations involved in laboratory tests, but it is enough to illustrate the contention that, if the results of biological toxicity

trials are to be applied to practice, consideration be paid to the physico-chemical properties of the spray or dust.

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IV. FUNGICIDES SUITABLE FOR FOOD PRESERVATION

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Innumerable substances possess fungicidal properties. Whether or not any of these substances can be usefully employed to prevent the growth of fungi in any particular instance depends not on their toxicity alone, but also on other properties such as solubility, taste, colour and smell. Consider, for example, the fungicides which are employed in a variety of ways in food preservation. Hypochlorites and formalin are used to sterilize storage rooms and grading machines and packing sheds. Benzoic acid or sulphur dioxide is added to certain foods to preserve them. Oranges are sometimes dipped in a solution of borax or sodium bicarbonate in order to reduce the incidence of rotting by green rot. Carbon dioxide is sometimes added to ships' holds to delay the onset of mouldiness and sliminess of meat carried in them. It has been suggested that certain fungicides might with advantage be added to the paper in which fruit is wrapped to prevent the onset or the spread of rotting. The compounds used in these various ways all possess the property of retarding or preventing growth, but otherwise the properties which make them useful are very different.

Because usefulness depends on the combined effects of a variety of factors, it is generally agreed that the advantage of using a fungicide in any particular instance can be tested only by direct experiment. Preliminary laboratory tests of substances can be undertaken to find:

(1) Their action on germination and growth of fungi or their power of killing, i.e. their fungicidal action.

(2) Their solubility, volatility, and other properties which may be of importance.

From the results of such tests, it is usually possible to decide what substances are obviously unsuitable and what substances may be suitable for further trial for the purpose for which they are wanted.

Laboratory technique for evaluating fungicidal properties

The type of test undertaken to find how fungicidal a substance is, is usually of the simplest order. This is to be regretted, for certain qualitative differences in the manner in which growth is re-

duced in the presence of inhibitors are often of importance in determining choice of fungicides.

To discover the effect of a substance on growth, it is necessary to measure germination or growth in the presence of increasing amounts of the substance. The difficulties in doing this are twofold.

There is the difficulty of knowing what criteria of germination or growth to adopt; and there is the difficulty of technique of carrying out the experiment.

The difficulty of knowing what criteria of germination or growth to adopt is due to the fact that substances may affect the course of germination by:

(i) decreasing the number of spores which germinate,
(ii) increasing or decreasing the latent period,
(iii) decreasing the rate of elongation of the germ tubes;

and the course of growth by:

(i) decreasing the rate of radial spread,
(ii) decreasing the density of the mycelium,
(iii) altering the final yield.

Moreover, these aspects of germination and growth may be affected differently as can be seen when one compares the effect, for example, of temperature, alcohol, carbon dioxide, oxygen and carbon monoxide on growth. For these reasons, any single criterion of growth is not sufficient to give adequate information about the action of a fungicide.

No one, I think, would suggest that the effect of temperature on the germination and growth of a fungus was fully described if the temperature at which 50 per cent of a sample of spores of the fungus failed to germinate was given. Yet one frequently finds some such value as the concentration of a substance needed to inhibit the germination of 50 per cent of a sample of spores claimed as a measure sufficient to describe the action of a fungicide. These points have been considered before (1).

The difficulties of technique include the choice of medium and the maintenance of the required condition. The following method has been found most suitable for studying the effects of soluble non-volatile inhibitors.

A sheet of squared paper is placed on a smooth level bench in a constant-temperature room, and covered with a sheet of glass. Petri dishes, 9 cm. in diameter, are set out in regular order on the glass, and into them are placed 10, 9·9, 9·8, 9·7, etc. c.c. of a liquid nutrient medium (2 per cent malt). To the dishes are then added 0, 0·1, 0·2, 0·3, etc. c.c. of the solution of the fungicide, ten to twenty times as strong as that necessary to prevent growth. (Preliminary experiments may be necessary to find out what concentration of fungicide is required to prevent growth.) In this way, a series of solutions containing increasing amounts of the substance is obtained.

Into the centre of each dish is placed a small piece of porous pot, previously sterilized and then inoculated with a small drop of a suspension of spores of the fungus. The spores germinate and the hyphae grow out regularly in every direction to form a circular mycelial mat, just as when growth is on a solid agar medium. The increase in size of the diameter of the colony can be measured by the aid of the squared paper. Care is required, especially in the early stage of growth, in examining and measuring the colonies, or the tips of the hyphae are thrown out of position and growth becomes irregular.

It is not always possible to make solutions ten to twenty times as strong as those which inhibit growth, and in such instances, some slight modifications are needed in making up the culture solutions in the dishes. For example 5 c.c. of a stronger culture medium may be added to each dish, and then 0, 1, 2, 3, 4, 5 c.c. of fungicidal solution and 5, 4, 3, 2, 1, 0 c.c. of water to make up to 10 c.c. in each dish.

The advantages of using liquid cultures are many. The solutions can be made up from two stock solutions sterilized separately. If there is any danger of the solutions in any of the Petri dishes changing in composition or *p*H, owing to the growth of the fungus, they can be sucked off daily and replaced by new solutions of the required composition. What is more, the composition of the solution can be changed after growth has started. It is usually advisable to buffer the nutrient solution with phosphate or phthalate buffers. When the diameter is plotted against time, a straight line is obtained. The slope of this line—the rate of increase in size of diameter—is generally the most suitable measure of the rate of growth.

How the manner in which a fungicide retards growths may affect its value as a means of preserving food materials

Graph I shows the effect of temperature and Graph II the effect of alcohol on the growth of a fungus.

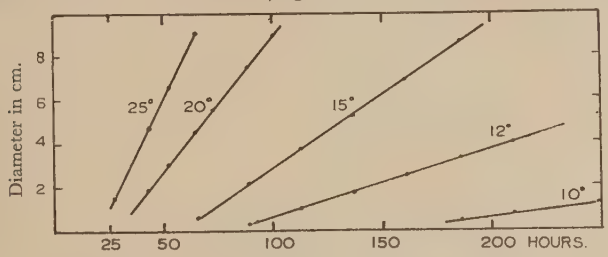
Lowering the temperature has two effects. The germination and early growth is delayed, and the rate of subsequent growth is decreased. The presence of alcohol decreases the rate of growth, but does so without any, or only a slight delay of germination.

The action of some substances (CO_2 and NH_3) is somewhat similar to that of temperature. The action of other substances (SO_2 and benzoic acid) is similar to that of alcohol.

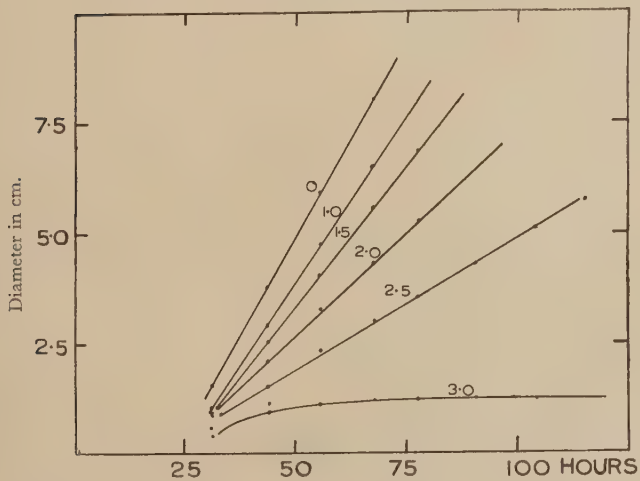
Thus the time taken for a fungus to grow to a definite size is altered to various extents by the presence of different inhibitors. The importance of this in food preservation is as follows: When food is stored, the length of time it can be kept (storage life) is of first importance. The time for food to become obviously mouldy is in certain

instances about the same as that required for the fungus to grow to 1 cm. in diameter, on a nutrient agar medium kept under the same conditions as the food. Now, whereas by decreasing the temperature from 25 to 10° the time required for the colony to grow to 1 cm. in diameter is increased from 25 to 240 hours, the effect of adding alcohol is to increase the time from 25 to not more than 40 hours.

By lowering the temperature sufficiently, or by raising the concentration of alcohol sufficiently, growth can be inhibited completely,



Graph I. Growth of *Trichoderma* at different temperatures

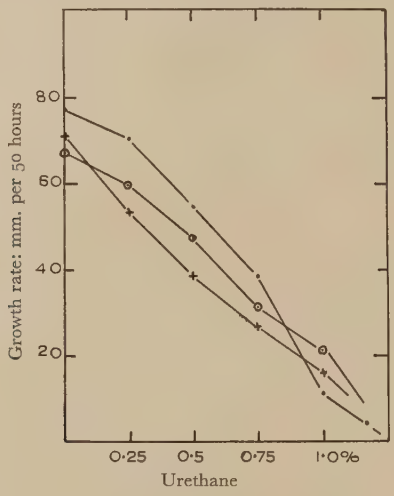


Graph II. Growth of *Trichoderma* in the presence of alcohol. (Parts per 100)

and the effect as far as mould growth is concerned is the same. But, except when conditions are so arranged completely to retard growth, the advantage of using low temperature or substances having a similar action, rather than alcohol (or one having a similar action) is obvious—though generally unrecognized.

The effect of the medium on the fungicidal properties of substances

The fungicidal properties of a substance often depend on temperature and the medium in which it is acting. One factor which often has a marked effect is the acidity of the medium.



Graph III. Growth of *Trichoderma*. ⊙ *p*H 4·0, • *p*H 5·0, + *p*H 6·0

The influence of *p*H on the action of three inhibitors is illustrated in Graphs III, IV and V, in which the rate of growth (as measured by rate of linear spread) is plotted against the concentration of the inhibitor.

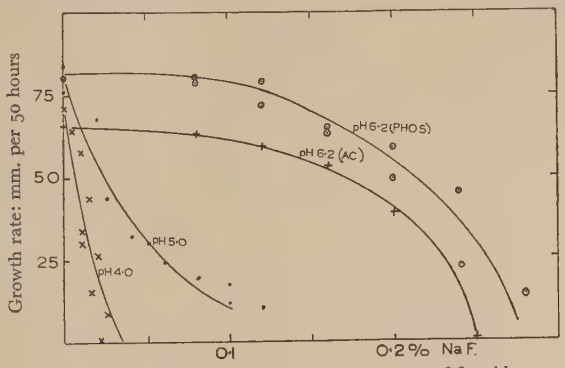
Graph III shows that the retarding effect of urethane on growth is directly proportional to the concentration and within wide limits unaffected by the *p*H of the medium.

Graph IV shows that the retarding effect of sodium fluoride is much more pronounced in acid than in alkaline solution, and that the relation between concentration and inhibition also varies with the alteration in the acidity of the medium.

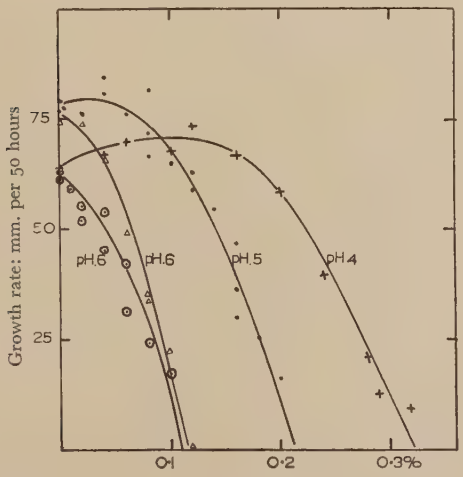
Graph V shows that the retarding effect of borax is more pronounced in alkaline than in acid solutions, and that the relation between concentration and inhibition varies as the acidity of the medium alters.

These figures suggest that in choosing a fungicide, some consideration of the acidity of the medium in which it is to be used is necessary,

and also that by suitably modifying the acidity of the medium, the efficiency of some fungicides can be increased. For example, when a substance such as urethane is used, no consideration need be paid to



Graph IV. Growth of *Trichoderma* in presence of fluoride



Graph V. Growth of *Trichoderma* in the presence of borax

acidity. But it might be profitable when sodium fluoride is used to make the medium more acid and use a smaller concentration of fluoride, than use a higher concentration of fluoride while leaving the acidity unchanged. Similarly when borax is used it might be profitable to make the solution more alkaline and use a lower concentra-

tion of borax, rather than increase the strength of borax and leave the acidity of the medium unchanged.

These simple examples show that detailed knowledge of fungicidal properties often suggests ways of improving fungicidal action.

However, when one tries to discover from published results how the action of fungicides is affected by various factors, one finds, strange though it may sound, that the details of the action of very few substances are at present known.

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V THE LABORATORY TESTING OF WOOD PRESERVATIVES

By W. P. K. FINDLAY
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The laboratory tests upon wood preservatives have been mainly of three types:

(1) Tests of toxicity in artificial agar media.

(2) Tests of toxicity in wood blocks.

(3) Tests of permanence, e.g. volatility and leaching tests on treated wood blocks.

In this discussion only the first two types of tests are referred to.

In the past so-called agar or Petri-dish tests have been extensively used, particularly in the U.S.A., for evaluating the toxicity of wood preservatives. They give clear-cut results which can be repeated if carried out under standard conditions. Continental workers have criticized the use of agar medium and have developed the wood-block test. Results obtained with experiments on wood are liable to vary and the experimental error may be high, but results in agar tests which may appear to be concordant can lead to very erroneous conclusions, since the reactions between an antiseptic with agar and with wood may be utterly different.

We now regard the agar test solely as a preliminary sorting-out test. It is useful for comparing the toxicities of substances of generally similar chemical composition, e.g. of various phenols, but is quite unsuitable for comparing the toxicity of substances of widely different composition, e.g. of creosote and of sodium arsenate. In carrying out tests in agar it is most important that the preservative and the medium should not be sterilized after mixing. Sterilized amounts of medium should be added aseptically to measured amounts of the preservative.

The total amount of nutritive medium and the total volume must be kept constant at each concentration. The end-point or toxic point should be expressed as the interval between that concentration at which growth just takes place and the concentration next above it in the series at which no growth takes place.

Except for such sorting-out tests, it is essential to use wood as a substratum for tests with wood preservatives. The species of wood used should be one which is readily decayed and has a low natural resistance to the attack of wood-destroying fungi. It should also be one which can easily be impregnated with a preservative. The sapwood of Scots Pine, *Pinus sylvestris*, and the outer wood of beech, *Fagus sylvatica*, are suitable woods.

The most useful figure for comparing the efficiency of wood preservatives is the minimum quantity of preservative which prevents all attack by certain organisms. This toxic point should be expressed as amount of preservative per unit volume of wood; it is useful also to give the strength of solution used to attain this concentration in the wood. The method of applying the preservative must be such that there is a uniform distribution of antiseptic. The most satisfactory method of achieving this is to impregnate thoroughly under vacuum, the blocks which previously have been sterilized and dried in an oven at 100° C., the samples being weighed before and after treatment to determine the absorptions obtained. Water soluble substances are applied in aqueous solution, oily materials in a suitable solvent such as petroleum-ether or acetone. After treatment with an aqueous solution the moisture content of the samples must be reduced to the point most suitable for fungal attack, i.e. to about 40 per cent of the oven-dry weight. Where an organic solvent has been used this must be completely evaporated away. This is accomplished by drying the blocks in an oven provided with forced ventilation at a temperature not exceeding 50° C.

The blocks are exposed to infection by placing them in contact with cultures of certain wood-destroying fungi growing on agar medium in special culture flasks. They are incubated for four months at 22° C. and kept in a moist condition by the addition of water to a reservoir in the neck of the culture flasks. At the end of this period the blocks are removed from the cultures, and examined for signs of fungal growth. They are freed from any superficial mycelium, then oven-dried and re-weighed. An indication of the amount of decay if any, which has occurred, is given in the loss in dry weight (initial dry weight – final dry weight), which is then expressed as a percentage of the original dry weight; allowance being made where necessary for the weight of preservative absorbed. In carrying out wood-block tests reliable results may be obtained but precise results cannot be expected.

A number of test fungi must be used since the wood-destroying fungi are not uniformly resistant to antiseptics. A fungus may be resistant to one substance and sensitive to another, for instance *Fomes annosus* is resistant to creosote and sensitive to zinc chloride, *Coniophora cerebella* is resistant to zinc chloride and very sensitive to creosote. At least three test fungi must be used, these should be:

(1) Active wood destroyers.

(2) Resistant to fungicides.

(3) Not unduly sensitive to slight variations in environmental conditions.

Lentinus lepideus Fr., *Coniophora cerebella* Pers. and *Poria vaporaria* (Pers.) Fr., are suitable for tests on pine, and *Polystictus versicolor* (Linn.) Fr. for tests on beech.

Summarizing the important points to be considered in making laboratory tests upon the toxicity of antiseptics for the preservation of materials:

(I) The tests should be made with pure cultures of the organisms against which it is desired to protect the material. With wood preservatives a number of test fungi should be used.

(II) The medium used must be the material which the antiseptic is designed to protect, in as nearly as possible its normal physical condition.

(III) Conditions for the growth of the injurious organism should be optimum.

VI. SEED PROTECTION

By W. A. R. DILLON WESTON
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In horticultural and agricultural practice the fungicides in use may be divided into three major groups: (*a*) fungicidal solutions, emulsions or suspensions, (*b*) fungicidal gases, (*c*) fungicidal dusts. It would be difficult, and probably impossible, to devise any one method of testing for such diverse phases. Although it is relatively easy to test simple soluble inorganic and organic solutions against a given fungal spore or spores when these are not in contact with their hosts, it cannot be inferred that the values so obtained will produce corresponding values in the field.

It would seem clear that laboratory technique for the evaluation of fungicidal values should be regarded as a rather close-meshed sieve to sort out probable fungicides from improbable ones. In such a sifting it may well be that evidence will be obtained that will indicate

relationships between chemical and physical properties, and fungicidal values and phytocidal effects.

It is the purpose here to discuss particular types of fungicides, viz. those that are used for the disinfection of seed. The disinfection of grain to prevent seed-borne disease is a well-established practice of plant hygiene and needs no comment. The methods employed, however, may alter. In recent years many farmers have changed from the wet treatments to dry, the latter consisting of disinfectant dusts that contain salts of mercury as their fungicidal basis. These dusts—complicated in chemical and physical properties—are now widely used, and by many are considered to be an advance on the older wet treatment, since, when correctly applied, they give control of Leaf Spot of oats and Leaf Stripe of barley—two diseases that were not adequately controlled by a wet treatment such as a solution of the gas formaldehyde in water. Moreover they are of value in the treatment of certain seeds other than cereals and better stands are sometimes established by the use of such treated seed. It is here that a slight digression is necessary. Laboratory tests are specific against a given organism but the seed when planted is in an environment in which it is in competition with a large and variable soil flora and fauna. To be effective the fungicide used must be capable of killing any disease organism adhering to or slightly penetrating into the seed, and also confer upon it *subsequent protection*. It is the analogy of Listerian practice that was founded on Pasteurian principle.

At Cambridge an investigation has been in progress for some years on seed protection, particularly on disinfectant dusts that contain salts of mercury, and a large range of these has been tested. As such dusts contain not only a fungicidal salt but a carrier for this, it is clear that *at least* two phases must be considered, i.e. the salt and the filler, and the chemical and physical properties of both must be known. Since these are two variables it does not follow that if a fungicide *A* has a greater value than a fungicide *B*, that a dust containing *A* is more potent than *B*.

Although theoretically—knowing the high toxicity of the salt—one might assume that a dust containing 5 per cent of mercuric chloride would be a good seed disinfectant (if it were practical to use it), this is not necessarily so, for when such a dust was used to treat infected oats the reduction in the infection of Leaf Spot was from 24 to 12 per cent only. The control, however, with Bunt contaminated wheat was better, the reduction being from 21 to 5 per cent.

Somewhat similar parallels could be drawn from a large range of inorganic mercury salts that we have tested. In this connexion too it may be of interest to record that when spores of *Tilletia* were exposed to the vapour of ethyl mercuric iodide this appeared to exert a lethal action. When, however, a dust containing ethyl mercuric iodide was

used on Bunt contaminated wheat sown in the field there was very little control of the disease, although the same dust gave a good control of Leaf Spot of oats. The sodium salt of hydroxy-mercuri-dibrom-fluorescein, although a well-known bactericide, gave little or no control of Leaf Spot of oats.

Observations such as these suggest that although some of the inorganic and organic mercury salts are specifics for certain seed-borne diseases, it does not follow that they are a panacea for all of them; moreover they indicate that laboratory evaluations, if unsupported by small-scale field tests, may be misleading.

It was for such reasons as these that we have held the intrinsic test for fungicidal values to be the disease control that is obtained on small-scale replicated and randomized field plots. Although biological laboratory work has served our purpose it has been in the field that our most useful records have been obtained. In this type of work a large laboratory garden is essential, and in the early part of our investigation we relied exclusively on this. In our preliminary trials we used the following technique. Dusts were prepared containing most of the known inorganic compounds of mercury and a selection from the organic compounds and these were compounded with the same filler. 5 and 10 per cent of the appropriate salts was used in these dusts and they were employed to control Leaf Spot of oats. The dose was at the rate of 2 oz. to the bushel of grain and the dusting operation was carried out for a period of 1 min. Each plot consisted of a rod row and each of these was replicated five times. Certain proprietary dusts were included for the purpose of comparison.

As the resulting estimates were made by recording the disease present in two hundred oat seedlings per plot there was therefore a total count of one thousand per treatment. In these preliminary experiments the plots were not randomized, but each treatment was spaced at regular intervals throughout the field and every tenth plot was an untreated one. Although this procedure has varied slightly a somewhat similar one has been adopted when investigating the control of other diseases and also in the various problems that have arisen during the course of the work. In several of the later trials eight replicates have been made and these have been randomized. In the main trials we have tried to assess the relative fungicidal values on a total count of not less than one thousand seedlings or ears per treatment. The results from some of these experiments we have already given (1).

The laboratory investigation was concerned with solutions of pure salts at known concentrations—in particular those that could be represented by the general formula *R*-Hg-*X* where *R* was a hydrocarbon and *X* an acidic radical. While at first sight the number of possible variants of *R* appears great, practical limitations such as

stability and methods of synthesis reduce the number to the following four:

Tolyl	C_7H_7	Ethyl	C_2H_5
Phenyl	C_6H_5	Methyl	CH_3.

This work was not extensive and we made no attempt to devise new methods to test existing seed disinfectants or to elaborate technique for a laboratory testing of fungicidal values. The objects of this investigation were primarily to obtain evidence on the relationship of toxic action to chemical constitution and to obtain information on the phytocidal effect of certain salts. The method we used was a simple one. In effect it was to determine the minimum concentration of the compound that would give the maximum control of the disease organism, i.e. *dosis curativa*, and to determine then the maximum concentration that the seed could tolerate, i.e. *dosis tolerata*. The latter was obtained by germination tests carried out at the Official Seed Testing Station, Cambridge. In our preliminary experiments watch-glasses containing Dox's medium were lightly flooded with a suspension of the spores of *Tilletia caries* and were exposed for varying periods to the vapours of the particular compounds. The amount of the spore suspension used and the period of exposure to the vapours was constant for a particular series of experiments. Rapid drying out of the medium was retarded by superimposing watch-glasses lined with unsized and moistened paper. Later, the percentage kill was determined by microscopic examination; and, subsequently, natural fungal and bacterial contaminations that developed on the treated media were noted, and the extent of these assessed and recorded.

Somewhat similar tests were made with solutions, chiefly with those containing methyl compounds. Spores of different species of *Ustilago* were used, since these germinate within twenty-four hours, whereas those of *Tilletia* take several days. A suspension of these in distilled water was added to glasses containing either Dox's or potato agar medium and the appropriate salt solution at a known concentration was then added.

A range of salts was also tested against the organism causing black-arm of cotton, *Bacterium malvacearum*. The technique employed was to take 5 c.c. of bouillon and glycerine, add 5 c.c. of a suspension of the organism in distilled water and then 5 c.c. of the appropriate concentration of the fungicide concerned. The point at which the organism was inhibited or killed was determined by the turbidity of the suspension or by reinoculation into sterile bouillon. The bactericidal activity of some of the compounds was also made by Rideal-Walker carbolic coefficient tests.

As some of the compounds we are using are volatile and are fungicidal in very small quantities, we are now trying to devise some method for estimating the relative fungicidal value of minute amounts

of them. The method that we are at present experimenting with is as follows: A small bulk of seed is dusted with a given quantity of the compound. The number of seeds per pound is known, consequently the amount of chemical per seed can be estimated. In this way minute quantities of the compound can be obtained and tested. A number of seeds so treated are then placed in a litre bottle in which is a small beaker containing a given quantity of water. A glass slide treated with potato agar on which spores of *Ustilago* are present is then suspended from the cork that seals the bottle. After twenty-four hours the glass slides are examined to see if the spores have been killed.

I have made no reference here to the results that have been obtained by these methods of investigation, or to those qualities—other than fungicidal efficiency—that are desirable in modern seed disinfectants, as it may be that such factors do not come within the scope of this symposium.

REFERENCE

(1) Dillon Weston, W. A. R. & Booer, J. R. (1935) "Seed disinfection. I. An outline of an investigation on disinfectant dusts containing mercury." *J. agric. Sci.* xxv, 628.

VII. SEED PROTECTION

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From 1929 to 1931 a number of experiments was carried out in Northern Ireland with the object of determining the most satisfactory treatment for securing the control of Smut diseases in the oat crop. Among the materials used for the purpose of seed disinfection were proprietary organic mercurials which at that time had not been placed on the market. The results obtained from these experiments showed that whereas formaldehyde gave almost perfect control of Smut diseases, the organic mercury compounds used were superior as general disinfectants against seed-borne diseases. As, at the same time, these compounds proved to be very little inferior to formaldehyde in securing Smut control and as there appeared to be no risk of seed injury attendant upon their use, it seemed probable that there was every likelihood of their being of considerable value in agricultural practice. To-day the use of these compounds is becoming widespread with the result that the plant pathologist whose duty it is to advise as to the most suitable measures to employ in securing the prevention of crop diseases is faced with one more problem, viz. he must be able to distinguish clearly between the products which are efficient and those

which are not. For the time being at any rate the advent of this new type of proprietary seed disinfectant has complicated the issue, and to put the matter bluntly, an improvement in the methods (or the devising of new methods) by means of which the values of fungicidal materials may be assessed as rapidly and accurately as possible is urgently needed. Biological technique only has been the subject of investigations in Northern Ireland and no attempt has been made to approach the question from chemical and physical standpoints. The object of the work has been to assess accurately the value of a fungicide by testing it against the disease which it is desired to prevent.

Helminthosporium disease of oats is common in Northern Ireland, and it was decided to attempt to devise a suitable technique for the testing of fungicides for the prevention of this disease. The problem was first approached by carrying out carefully planned field trials. Towards this end, seed known to be heavily infected with *Helminthosporium* was sown in drills at a standard rate at the normal sowing time, and when the seedlings had reached the optimum stage of development they were examined for the incidence of the primary phase of the disease. The results obtained were useful but lacked precision, and it became clear that several seasons' work would be necessary before really accurate data could be obtained. Furthermore, other factors arose which indicated that a more precise method applied under carefully controlled conditions was highly desirable in addition to field trials. In brief these factors may be cited as follows:

(*a*) A large number of treatments were used and it was found impracticable to lay down the number of plots necessary to ensure the statistical accuracy of the results obtained. The employment of an accurate small-scale technique would at least allow a number of inefficient materials to be discarded in the preliminary stages.

(*b*) It is important that the counting of diseased seedlings be concluded before the onset of the secondary phase of the disease. The undertaking of field experiments on too large a scale makes this difficult, while a period of inclement weather during the critical period may impose a further difficulty.

(*c*) The extent of the incidence of the disease varies largely from season to season in accordance with the weather conditions experienced.

Before attempting to devise a small-scale method of testing it was decided to adopt some definition of the ideal seed disinfectant and the following was chosen:

The ideal seed disinfectant shall be that which will secure complete control of a seed-borne disease *when the crop is grown from heavily infected seed under conditions designed to produce the maximum incidence of disease.* It will exert no adverse effect upon the crop, be non-poisonous

to animals or man, and its use will entail a minimum expenditure of money and labour.

The adoption of the conditions laid down by this definition involved a considerable amount of preliminary work in order to determine the conditions under which seed oats heavily infected with *Helminthosporium* will produce a crop showing the maximum incidence of disease. The seedlings were raised in round cake tins filled with soil and each capable of accommodating a hundred plants. An equal quantity of soil was weighed into each tin and the seeds were sown at a constant depth and equally spaced. Each seed was dropped from forceps into the hole prepared to receive it in order that the sowing process should remove as little of the disinfectant as possible. After sowing, the same weight of covering soil was used for each tin. The plants were grown to such a stage as to ensure that the primary phase of the disease had developed to its fullest extent in the crop. By means of this method the influence of soil, moisture, and temperature upon the incidence of the primary phase of the disease in the crop was investigated. Two types of soil were used, one light and sandy, the other a heavy loam. As no large greenhouse with adequate temperature control was available, the effect of temperature was studied by making five different sowings during the period from 1 March to 1 May, which represents the extreme sowing range for the spring oat crop. Two recording thermographs were run in conjunction with the experiment and an average temperature for each sowing was calculated from records made at intervals of two hours throughout the experimental period. Three degrees of soil moisture were used, one representing a very wet soil, the second a soil with a medium moisture content and the third a very dry soil. The access of rain to the tins was prevented by carrying out the work in an outside verandah built against a wall and with a roof sufficiently wide to prevent the rain from entering. In order to ensure the constant moisture content of the soil in the tins during the experimental period, each tin was watered, every other day, until its weight was the same as that recorded at the commencement of the experiment. The time of the experiment varied for the various sowings from sixty to thirty days according to the temperature and moisture conditions prevailing.

In brief, the results from these experiments have shown that those conditions which favour the good growth of the oat crop lead to a minimum incidence of *Helminthosporium* disease. The pre-emergence phase of the disease is pronounced under conditions which make for the slow germination of the seed and growth of the seedling; it was particularly marked when low temperature conditious prevailed. The minimum incidence of disease occurred under conditions of wet soil and high temperature. In dry soil the disease was severe even under

conditions of high temperature. Soil type is probably the least important of the three factors investigated, light soils tending to favour the incidence of the disease more than heavy soils.

For testing purposes a good medium loam is satisfactory. The temperature and moisture conditions should be such as will lead to a maximum incidence of the post-emergence phase of the disease and a minimum of the pre-emergence phase. An average temperature of from 8 to 9° C. throughout the experiment provides those conditions, and in Northern Ireland for the two seasons 1934 and 1935 they have been obtained by commencing the test during the second week of March. As wet soil does not encourage the development of the disease to its fullest extent, such soil is not used for testing purposes. Both moderately moist soil and dry soil are used, the moderately moist because it approaches natural conditions and the dry because it has been found that dust disinfectants tend to be less effective under dry soil conditions, which must therefore be included so that the efficiency of any disinfectant can be correctly assessed.

These necessary conditions for testing were first investigated in the spring of 1934 and results obtained from repeat experiments made in 1935 fully confirmed the findings. A series of comparative tests using a large number of fungicides was carried out in 1935 and very satisfactory results were obtained.

Continental methods

In the summer of 1934 I visited the Continent and was privileged to inspect the work being carried out at the State Biological Institute, Berlin, in connexion with the official testing of fungicides and insecticides. The following is a summary of some of the methods and technique employed with regard to the testing of seed disinfectants.

The German Plant Protection Service publishes an official list of approved plant protection substances. To qualify for inclusion in this list a material must satisfactorily pass a series of official tests. Materials which can be prepared by any person following given instructions or to the use of which objection may be taken in the public interest, cannot be included. The tests to which materials are subjected fall into two groups: (i) The Preliminary Tests, and (ii) The Main Tests.

The preliminary tests

These are carried out at three of the central stations in Germany, the manufacturer being allowed the choice of station.

The *seed grain used for the tests* must have a normal water content (13–14 per cent). For Bunt, clean seed wheat is used and is artificially infected with *Tilletia* spores at the rate of 0·2 g. per 100 g. of grain.

For Loose Smut in oats two seed samples from different sources are used. At least one of these samples is taken from a crop the plants of which were infected at flowering by dusting them with spores of *Ustilago Avenae*. Seed rye must not be infected with common moulds to an extent of more than 5 per cent.

Germination tests are carried out according to the methods employed at the official seed-testing station. The samples used for this purpose must be the same as those intended for use in greenhouse and field trials. Special attention is paid to the question of seed injury and where this is suspected soil tests are carried out in addition. Tests carried out at temperatures of from 6 to 10° C. are made when possible, in addition to those made at from 18 to 22° C.

Laboratory methods used for the determination of fungicidal efficiency in the case of Bunt in wheat vary according to the method of disinfection employed. For the *steeping* process *Tilletia* spores are directly treated with the fungicide and then tested for viability at stated intervals after they have been plated out on moist soil contained in Petri dishes. Tests for the *sprinkling*, *short-steeping* and *dusting* processes are made by treating artificially infected grain which is then sown on the surface of moistened soil similar to that used for the steeping process. The *Tilletia* spores present are examined for viability at stated intervals. The methods used for Stripe diseases of cereals caused by *Helminthosporium* spp. are similar to those employed for Bunt in wheat. For *Fusarium* disease of rye, tests are made in the *greenhouse* by planting disinfected seed in soil in wooden boxes. The spread of the mycelium in the soil is prevented by enclosing each seed in a narrow glass cylinder (6 × 2 cm.) which is pressed into the soil. Counts of diseased seedlings are made at stated intervals.

The *drilling capacity* of disinfected grain is determined by the use of an experimental drilling machine. Three estimations are made of the amount of grain which passes through the machine after it has made one hundred revolutions.

Field experiments are carried out in addition to laboratory tests.

The *hygroscopic nature* of dust preparations is determined by exposing a known quantity of the dust in a moisture-saturated chamber. The water increase by weight is estimated after a period of seven days.

Disinfectant dusts are tested for their *action upon iron*. Layers of the dust are placed upon small iron plates, some of which are then put in a moisture-saturated chamber and others in the laboratory, the moisture of the air of which is determined. The rust-promoting properties of the disinfectant are assessed after a period of three days.

The main tests

Materials which successfully pass the preliminary tests are recommended for the main tests, the chief object of which is to determine the efficacy of the preparation in practice under different weather and soil conditions. The main tests are carried out by at least eight and not more than twelve of the central stations for plant protection, and are made in much the same way as the preliminary tests. Only those concentrations which have proved to be effective in the preliminary tests are used in the main tests.

After the completion of the main tests the results are considered by a judging committee which decides whether the material shall be included in the official list. For materials giving unsatisfactory results and not admitting of a definite decision, the manufacturer is given an opportunity of furnishing his observations before a meeting of the committee.

When a material has been passed for inclusion in the official list, the manufacturer is required to sign an agreement which tends to safeguard the purchaser and among other things guarantees that no other products will be placed on the market under the trade-mark of the listed preparation.

So far as I am aware, the submission of material for official tests is on a purely voluntary basis and this seems to me to be rather a strong point in favour of such a scheme. Nobody is precluded from the manufacture and marketing of plant protective substances. Those who do so have the opportunity of submitting their products for official tests which, if successfully passed, allow it to be known that the material is officially approved for use at certain concentrations by certain methods for certain purposes of plant protection. This should mean a great deal if the work of official stations enjoys the confidence of the farming community.

Apart from the official work carried out at the State Biological Institute, work on an elaborate scale is conducted by the large industrial concerns engaged in the manufacture of plant protective substances. The Biological Institutes of the I.G. Farbenindustrie at Leverkusen (Cologne) and Hoechst (Frankfurt) have large well-equipped buildings with adequate field facilities close at hand. The testing work carried out by the scientific staffs of these Institutes closely approaches the lines followed by the Official Institute and results obtained by the station at Leverkusen are checked at Hoechst. I believe that many thousands of materials were tested before the present preparation recommended by I.G. Farbenindustrie for seed disinfection was marketed. While visiting these institutes I had the privilege of seeing tests being made with preparations in order to determine their efficiency for controlling the following diseases: Bunt

in wheat, Leaf Stripe in barley, Smut in oats, *Fusarium* disease of rye, Apple Scab and Downy Mildew of the vine. An entire laboratory was devoted to testing work dealing with fungicides for the prevention of timber decay. Apart from fungicides, insecticides and vermicides were receiving equally careful attention. From the few observations I was able to make, the relations existing between the staffs of the industrial institutes and that of the Official Institute, seemed to be healthy and of the happiest kind.

During the subsequent discussion Mr C. T. Gimingham referred to a general point touched on by Mr Marsh, and emphasized the importance of keeping in mind the precise object in view in working out the technique of laboratory methods of testing.

Methods may be required (*a*) for use in fundamental research on toxicity and (*b*) in testing commercial and other preparations. For the first purpose it is justifiable and necessary to aim at the highest possible degree of accuracy, but to attain this in biological test methods almost invariably involves a degree of elaboration and expenditure of time which is likely to disqualify the method for use in routine work. In devising methods for the second purpose it may be necessary to sacrifice some degree of refinement, and it will be agreed that it is likely to be waste of effort to use elaborate and very accurate apparatus unless it is also possible to have standardized and uniform biological material for the tests. In this connexion Mr Gimingham referred briefly to the methods adopted by the German Plant Protection Service for use in their official tests, some of which he had had an opportunity of seeing during a visit to the Institute at Dahlem last summer. He had formed the impression that at any rate so far as the methods for testing insecticides were concerned (and probably the same applied to the methods for fungicides) they had been designed with a very practical aim. Although giving satisfactory and reliable results for the particular purpose in view they would not all necessarily be suitable for (nor are they intended for) more fundamental investigations. Relative simplicity and ease of operation must not be lost sight of in considering methods for routine testing of commercial products.